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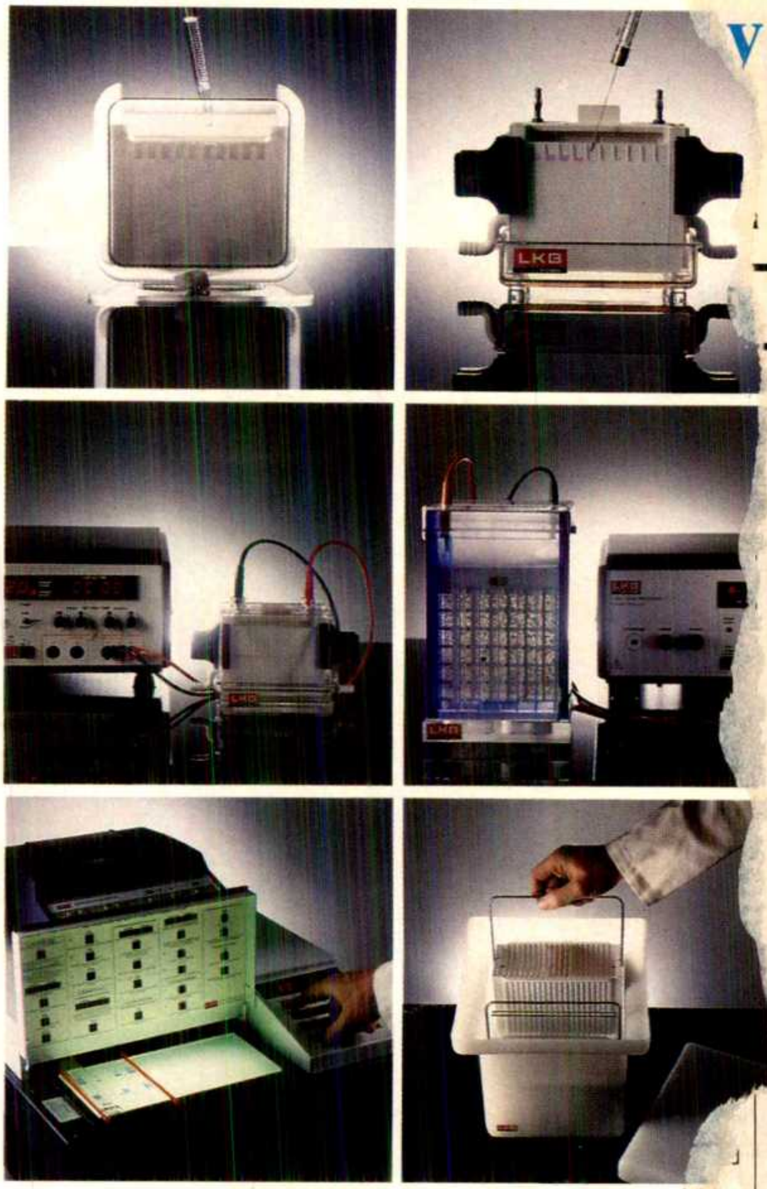

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ansforming growth factor type beta isolated from human platelets (hTGFβ) or from porcine platelets (pTGFβ1) is sequentially (first 50 a.a.) and biologically identical. Both give an ED_{50} of 0.1-0.4 ng on NRK (49F) cells when assayed as per Assoian *et al* JBC *258*, 7155, 1983, and 0.8-1.0 ng/ml on AKR (2B) after Tucker *et al* CAN RES *43*, p1581, 1983.

[2] pTGFβ2 is a structurally distinct second form of TGFβ (sequence homology 70% in the first 50 a.a. to hTGFβ/pTGFβ1). When assayed as above, it gives the same ED_{50}s on NRK and AKR cells. However, some cell lines can distinguish between pTGFβ2 versus pTGFβ1/hTGFβ.

[3] Human platelet derived growth factor h(PDGF) is assayed on Balb/c-3T3 and NIH-3T3 cells for H^3-thymidine incorporation as per Raines and Ross, Meth. in Enz. *109*, p749.

[4] Porcine PDGF is structurally very different from hPDGF (i.e. differs in M.W., subunit composition, N-terminus), and is biologically less active in thymidine incorporation assays.

[5] Both the acidic and basic forms of bovine fibroblast growth factor are isolated from brain and assayed for H^3-thymidine incorporation on NR6-3T3 cells after Gospodarowicz, *et al* JBC, *253*, p3736, 1978.

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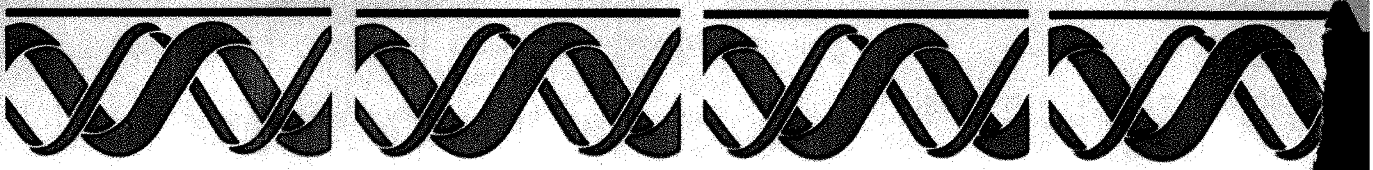

The 1987 Bio/Technolog

NEW DIRECTIONS IN PRODUCT

Gene Regulation ● Receptor Biology ● Protein Desig

How does a cell regulate the expression of complex genes? How does an expressed protein fold into a biologically active form? How can one modify a protein to increase its specific activity? How do pharmaceutically active proteins interact with their receptors? And how, in turn, do receptors regulate gene expression to start the cycle over again?

These are the open questions that every researcher *must* answer as he or she follows the trail to a commercial product or process. This January, a score of the world's leading researchers will talk frankly about their approaches—drawing on the experience that has helped guide enterprises like Amgen, Applied Biosystems, Biogen, Cetus, Genentech, Genetics Institute, Merck, Plant Genetics Institute, Smith Kline & French, Triton Bioscience, Upjohn, and the 200-company MIT Industrial Liaison Program, among many others.

The conference structure will offer ample time for discussion of practical R&D and process development problems with panels of acknowledged experts. The program is of interest to any biotechnologist trying to control expression levels in a culture process, produce pharmaceuticals in plants, treat cancer, immunize against AIDS, increase activity in established processes, improve a commercial enzyme, or solve *any* nuts-and-bolts problem.

CONFERENCE SCHEDULE

Monday, January 26
REGULATION OF GENE EXPRESSION

Plenary Address
George Poste, Smith, Kline & French Research Laboratories

The Replication and Transcription of Satellite Phage P4
Richard Calendar, University of California, Berkeley

Transcriptional Activation
Martin Rosenberg, Smith Kline & French Research Laboratories

Alpha Interferon: Synthesis and Mode of Action
Charles Weissmann, University of Zurich

Organ-Specific Expression of Chimeric Genes in Transgenic Plants
Marc van Montagu, State University of Ghent

Regulation of Soybean Seed Protein Gene Expression in Transformed Plants
Robert Goldberg, University of California, Los Angeles

The Immunoglobulin Gene Superfamily: Expression and Evolution
Leroy Hood, California Institute of Technology

Panel Discussion: Calendar, Rosenberg, Weissmann, Van Montagu, Goldberg, Hood.

Evening Delegates' Reception sponsored by New Orleans Chamber of Commerce

CONFERENCE SCHEDULE

Tuesday, January 27
RECEPTOR BIOLOGY: TOWARD NEW PHARMACEUTICALS

Receptor Regulation: An Approach to the Treatment of Hypercholesterolemia
Michael Brown, University of Texas Southwest Medical School

Antireceptor Antibodies: Probes of Viral Attachment and Use as Immunogens
Mark Greene, University of Pennsylvania

Structure and Function of the T-Cell Receptor
Susumu Tonegawa, Massachusetts Institute of Technology

Panel Discussion: Brown, Greene, Tonegawa

Control of Cell Behavior by the Epidermal Growth Factor Receptor Gene
Ira Pastan, National Cancer Institute

Functional Analysis of Growth Factor Receptor Tyrosine Kinases
Axel Ullrich, Genentech

The Interleukin-2 Receptor on Normal an Malignant Cells: A Target for Immunotherapy
Thomas Waldmann, National Cancer Institute

Panel Discussion: Pastan, Ullrich, Waldmann

Evening Delegates' Reception

New Orleans Conference:
AND PROCESS DEVELOPMENT

January 26-28, 1987 at the New Orleans Marriott

CONFERENCE SCHEDULE

Wednesday, January 28
REDESIGNING PROTEINS

Genetic Identification of Amino Acid Sequences Directing the Folding Pathway of the P22 Tailspike Proteins
Jonathan King, Massachusetts Institute of Technology

After the Cloning is Over: Redesigning Enzymes via Genetic Engineering
Charles Craik, University of California, San Francisco

A Biophysical Approach to Understanding Protein Secretion
Lila Gierasch, University of Delaware

Panel Discussion: King, Craik, Gierasch

Modifying Enzyme Activity and Stability
Gregory Petsko, Massachusetts Institute of Technology

The Recovery and Refolding of Biologically Active Oligomeric Proteins
Rainer Jaenicke, University of Regensburg

The Roles of Disulfide Bonds in the Structural Stability of Proteins
Ronald Wetzel, Genentech

Panel Discussion: Petsko, Jaenicke, Wetzel

HOW TO REGISTER

Your $395 registration fee entitles you to all three days of conference sessions and social functions, a book of abstracts, and a list of other meeting attendees.

Mail the registration form or a photocopy to:

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If the coupon has already been removed from this advertisement, write to the above address or call Diana Berger at 212/477-9600.

HOTEL RESERVATIONS

Bio/Technology has reserved a limited number of rooms at special rates for conference attendees. To reserve a room at the special conference rate of $78 per person, please contact:

The Marriott Hotel
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504/581-1000

TRAVEL

To obtain air tickets at special conference rates, please contact Aaron Krumbein at Consolidated Tours, 250 W. 57th St., New York, NY 10019. 800/228-0877. Or call Eastern Airlines directly, through the Convention Desk, 800/468-7022. Please refer to the Bio/Technology New Orleans Conference, reference number EZ1AP57.

ON-SITE REGISTRATION

Early registration at the New Orleans Marriott Sunday, Jan. 25, from 5 p.m. to 7 p.m. General registration Monday, Jan. 26 through Wednesday, Jan. 28, from 8 a.m. to 5 p.m.

REGISTRATION FORM

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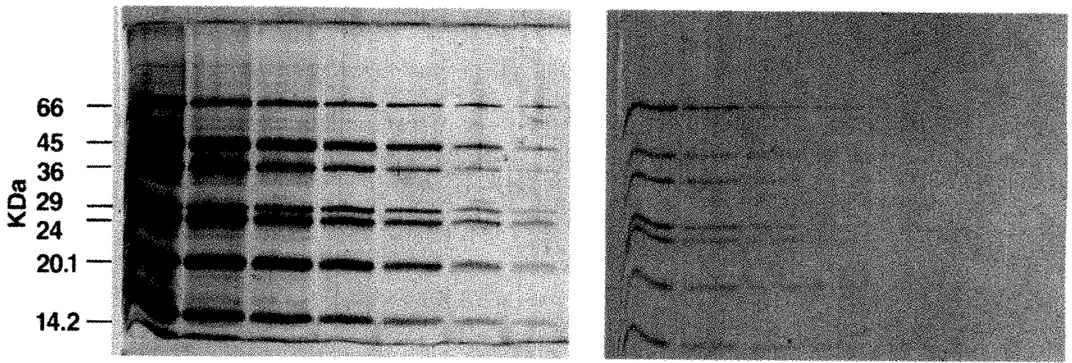

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15 January 1987

Volume 325

Explosion of a dispersed 1-tonne charge in Lake Baringo, Kenya, during the Kenya Rift International Seismic Project (KRISP 85). Seismic refraction data obtained from eight explosions along the rift axis are used by the KRISP working group (pages 239–242) to constrain the deep structure of this geologically important feature.

THIS WEEK

■ Scientific fraud

This week's *Nature* includes three articles dealing with some of the consequences of the now notorious case of scientific fraud which came to light in 1981, when Dr John C. Darsee, then a postdoctoral fellow at the Harvard Medical School who had previously been at Emory University, was discovered to have fabricated data for a series of research papers.

■ A Commentary "The integrity of the scientific literature" by Drs W. W. Stewart and Ned Feder, at the National Institutes of Health. (page 207)

■ A reply by Dr Eugene Braunwald, Hersey Professor of the theory and practice of medicine at the Harvard Medical School. (page 215)

■ A leading article (editorial) explaining *Nature*'s role and interest in the publication of the Commentary by Stewart and Feder. (page 181)

■ Transgenic rye

. . . pages 192 and 274

By injecting DNA into the flowers that will turn into grain on stalks of rye, plant scientists in West Germany have found a way to introduce new genes into a major cereal crop. In a News and Views article the methods currently available to transform plants genetically are discussed.

Last week's issue of *Nature* carried an incorrect issue number on the cover. It should have read No. 6100, and this week's is No. 6101.

■ Books

. . . page 203

Who was "The Griffin"?

■ Planet atmospheres

. . . page 197

A recent spacecraft encounter with Uranus has provided many new clues on atmospheric circulation.

■ The spider's web

. . . page 234

How does the solution extruded by a spider immediately turns into a strong silken thread.

■ Brain asymmetry

. . . pages 196 and 249

It is the left half of the brain of the house mouse that deals with sound according to experiments carried out by G. Ehret. So the specialization of the two halves of the brain that is so well documented in humans seems to have its origins at an early stage of mammalian evolution.

■ State-of-the-art

. . . pages 194 and 236

Colin Fyfe traces the development of solid-state NMR. Kirkpatrick and co-workers provide evidence for the power of this technique in structure determination.

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181 Fraud, libel and the literature

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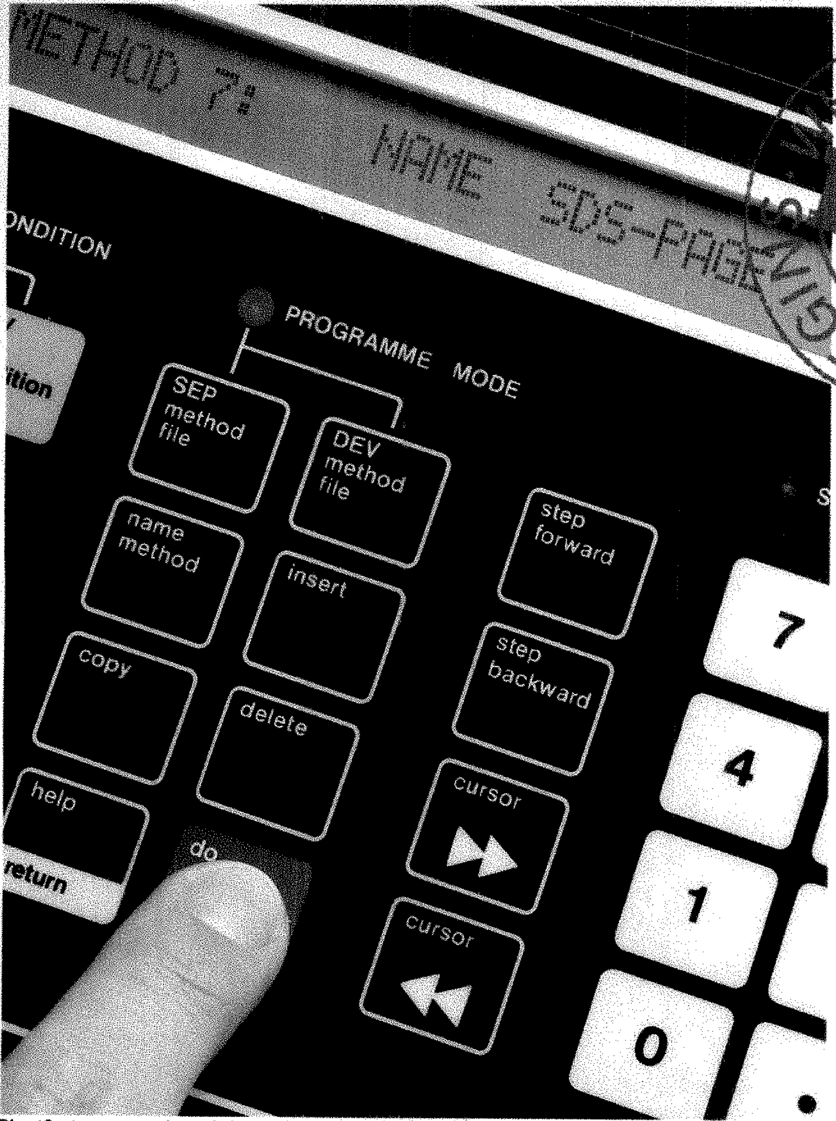

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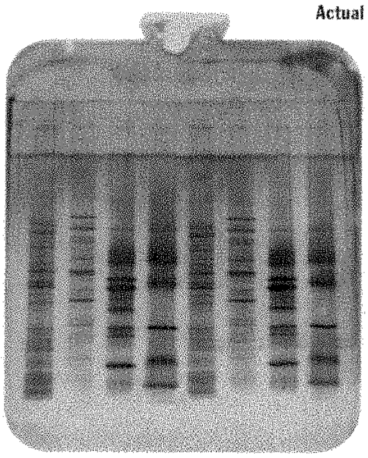
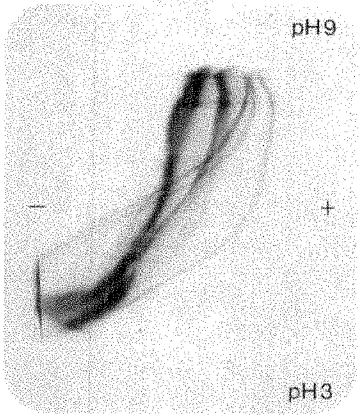


SDS-PAGE on PhastGel Gradient 10–15 with silver staining. Samples of meat and *E. Coli* proteins. FROM SAMPLE TO RESULT: 90 MINUTES.



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■ Comet Halley nucleus modelled ■ Is Earth's core iron? ■ Channelling and channelling radiation ■ Protein-based adhesive ■ Human chromosome jumping libraries ■ Cardiac potassium transport ■ Defective enzyme in Bloom's syndrome

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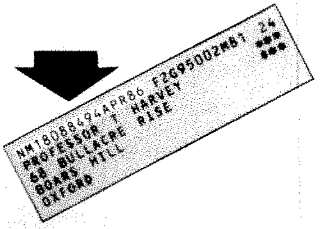

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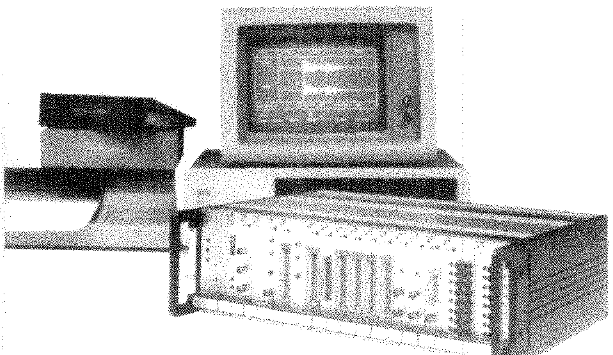

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nature

Fraud, libel and the literature

A much-publicized attempt to show that the scientific literature is flawed is not itself above reproach. What science needs is less, not more, solemn attitudes to professional publications.

The remarkable document on pp 207–214 of this issue should be read only in conjunction with the comment thereon by Dr Eugene Braunwald (pp 215–6) and the following explanation of *Nature*'s role and interest in this unusual affair. Drs Walter Stewart and Ned Feder are two tenured members of the staff of the US National Institutes of Health (NIH) who have carried out an analysis of the research reports and other documents published in the scientific literature by John C. Darsee, who was discovered in May 1981, while a postdoctoral fellow at the Harvard Medical School, to have been guilty of deliberately fabricating data. So much was quickly established by committees of investigation set up by Harvard, by Emory University (where Darsee had previously worked) and by NIH. Stewart and Feder have attempted, by the scrutiny of Darsee's prolific published works, to identify errors, inconsistencies and other flaws that are unconnected with the known falsification of data. The essence of their conclusion is that errors, necessarily subjective in definition, are abundant; this, they say, reflects on the care with which Darsee's coauthors discharged their responsibilities, as well as on the management of scientific journals by their editors and referees.

As will be seen from Braunwald's comment, the methods and the conclusions of this unusual study are disputable; no doubt there will be other responses of the same kind to some of the other illustrations in Stewart and Feder's short list of examples. One obvious difficulty is that, by sticking woodenly to the decision that they would base their study only on the published literature, not asking those responsible for explanations, Stewart and Feder have no doubt been led to count as errors some occasions when authors have merely failed to make their intentions clear.

Yet the document will inevitably raise in readers' minds important questions about the reliability of the scientific literature and of the role of the process of publication in the practice of science. Is the literature in general as flawed as Stewart and Feder claim their "sample of convenience" to be? And, if so, why, and what can be done about it? Many will say that the first assertion has not been proved, so that the second question does not arise, but that is too complacent an opinion. There are also important questions about the pressure to publish that need attention, independently of the analytical part of what Stewart and Feder have written. So why not consider those issues in their own right, without publishing a document whose accuracy is disputed?

Notoriety

The truth is that the document by Stewart and Feder has by now acquired a notoriety comparable with the Darsee affair itself. An early version was submitted for publication in *Nature* in 1983; two reviewers (outside the United States) took the view that the manuscript, while of absorbing interest, would require that certain editorial questions of principle should be decided before publication could be seriously considered. The paper was accordingly sent for comment to a dozen or so of the coauthors identifiable even from the anonymous list of Darsee's publications in the accompanying bibliography, three of whom replied through their attorneys with the opinion that publication would be defamatory, an opinion already given by *Nature*'s own legal advisers.

Litigious readers may note, at this stage, that, at least under British libel law, it could well be libellous to publish an innocuous version of a manuscript accompanied by the intelligence that earlier versions had been defamatory of still identifiable people. Readers should therefore know that what follows has been discussed with some of those concerned, who have agreed that it should be published so as to bring out into the open an issue of which Stewart and Feder have recently made much.

Replication

Thanks to the marvellous powers of word-processors, the first version of the manuscript was quickly followed by many others. At a meeting in September 1984 with two of the co-authors and their attorneys, the editor of *Nature* was left in no doubt that publication of any of the versions then extant would be considered defamatory, an opinion with which he agreed. At the same meeting, a number of constructive suggestions were made for modifications that might make a publishable manuscript. In the event, the need to transact normal business, and the fearsome volume of comments and counter-comments that had accumulated, meant that it was not until the near the end of 1984 that an edited version of the manuscript which *Nature* considered to be acceptable was being transmitted by telefacsimile from London to the authors at NIH. When only 26 of more than twice as many pages had made the transit, the recipient authors telephoned hotly to say that the changes made the manuscript unacceptably anodyne, and that it would be withdrawn; they were told that *Nature* would be prepared to reconsider a new version of the original amended along the lines suggested if they failed in their expressed intention of having the original published elsewhere. The version now published is derived, mostly by abbreviation, from one submitted a year ago.

These tedious details are relevant to present circumstances because of the way in which Stewart and Feder have injudiciously, during the past year, sought to argue that the threat of libel suits has been used to suppress an important comment on the integrity of the scientific profession. This was the essence of their case to a congressional subcommittee in the United States last year, in a series of statements to the *New York Times* (26 April 1986) and, more recently and more damaging, in a letter (accompanied by an earlier unexpurgated version of the manuscript) asserting that publication had been prevented by the threat of libel suits; that letter was sent to all 1,800 members of the US National Academy of Sciences as well as to many of the authors' correspondents on quite unrelated matters. At a guess, upwards of 2,000 people have been circularized in this way.

Integrity

The issue thus raised has an important bearing on the conduct of the scientific literature, which depends for its integrity on its accessibility and openness; in principle, all scientists are free to offer their contributions. Dissent from accepted theories is allowed and even welcomed. Novel ideas may be published for the sake of the stimulus they provide, but with no implied guarantee that they are in some absolute sense 'correct'. Moreover, authors are frequently made painfully aware by their experience that publications are hostages to fortune; it frequently occurs that the publication of a set of data and of a plausible hypothesis to account for them will stimulate others to produce further data whose effect is to contradict the hypothesis, to the chagrin of the original authors. Often, publications are found to contain self-vitiating errors, whose authors then have even redder faces. Such honest mistakes are an inescapable part of science.

So why does not this process of cut and thrust, personally hurtful to those concerned, require that scientific journals employ armies of lawyers vetting everything they publish? Because of the principle that, while it is a proper and necessary function of authors to correct what appear to be erroneous data or inferences in the literature in publications of their own, most keep to the convention (which is otherwise enforced by editors) that the identification of an error is not followed by the assertion that the perpetrator is a rogue, let alone by speculation about the motives that have engendered this condition. Stewart and Feder have complained that they could not publish what they chose because of "threats of libel". They have not understood that the unfettered right to publish scientific data does not equate with a right to denigrate others' characters.

For all this, what Stewart and Feder have written deserves close attention. At the very least, they have shown that a group of published papers do not provide a full explanation of the data recorded in them. Even if the "bizarre pedigree" they have found (see p.208) would be less puzzling if the ages given in the original paper were intended to mean something other than chronological age, there is nothing in the paper to suggest that simple readers would differ from Stewart and Feder in their interpretation of the paper. Similarly, while Braunwald's defence of the use of "historical controls" is entirely proper, it remains a fact that the procedure that should have been followed (Darsee cut corners instead) is not adequately explained.

Imprecision

How can imprecisions of this kind have found their way into published papers? Stewart and Feder are unforgiving in their opinion that Darsee's coauthors should have spotted them, and have got rid of them, but that asks a lot of flesh and blood. If the documents concerned were drafted in the first place by Darsee, often with the intention of rendering plausible a set of data derived from thin air by fabrication, it requires very little imagination to appreciate the difficulty of rooting out minor errors at source; those who spend time on committees know how much initiative rests with the one who writes the minutes. Stewart and Feder themselves appear to have spent a long time, assisted by the hindsight that Darsee was a fraud, scrutinizing just over a hundred publications. Braunwald also makes a cogent point in saying that the detection of the major error, the fraud, would have required the repetition of the experiments, defeating the objective of research by teams of people. But the general conclusion at which Stewart and Feder arrive, that coauthors should be more zealous in their scrutiny of what is about to be published in their names, anodyne though it may be, cannot be a bad prescription.

Coauthorship as such is a necessary consequence of the way that science is now practised, enabling people with different skills to work together on a single project; it is also capable of being abused. Stewart and Feder define as "honorary" those coauthors who have not been directly involved in the research reported in published papers. This is a restrictive definition, especially where the members of a research team include senior and less senior people. Most people, probably including Stewart and Feder, would accept the Braunwald position that a person whose role has been to conceive of a research programme, to raise the funds with which it is supported and to supervise progress, is legitimately a coauthor of the research. This is the standard relationship between academic researchers and their graduate students, for example.

On more general grounds, Stewart and Feder are right to insist that the literature is corrupted if its users cannot be assured that all the authors of a publication have played some essential part in its development. There is evidence, mostly anecdotal, quite unconnected with the Darsee case, to show that what they call "honorary coauthorship" is commonplace. In molecular genetics, for example, those who supply clones of some genetic material for use by others are often listed among the authors of the ensuing research. Some coauthorship is political in flavour, as when the prime movers in international projects cite sleeping partners as authors for the sake of continued opportunities to investigate otherwise inaccessible phenomena. The practice that the head of a laboratory should as a matter of course appear as an author of all its publications, still accepted as a convention in some Indian laboratories, for example, is rapidly on the way out and should be helped on its way by Stewart and Feder's article, which will remind those concerned of the risks they run. Some journals, responding to the *samizdat* publication of that article, have gone so far as to decide that future coauthors will be required to declare their full involvement in work sent for publication (a practice, interestingly, followed as a matter of course by groups of authors from the Soviet Union). But by itself, such devices will not end the abuse of coauthorship.

Publications

How common are errors of the kind that Stewart and Feder categorize? And how serious are they? Their own study, some of whose conclusions are disputed, concerns an exceptional sample of the literature. But, shocking though it may seem, the experience of those concerned with the management of the literature is that errors of all the kinds listed are far from being rare. Even when authors do their best, honest mistakes crop up repeatedly. (Scientists are too often indifferent to the plain truth that awkward or ambiguous phraseology is one of the most common reasons why scientific publications are misunderstood.) Luckily, as the world knows, most of these difficulties are cleared up by the informal correspondence between interested parties which is now as much a part of the process of science as the formal literature. More serious faults are less easily dealt with. Instalment publication is often used to magnify a laboratory's output and, as all journal editors know, there are also many occasions when authors are less than diligent in referring to the intellectual sources of their work. Ignorance is often part of the explanation, but ignorance can cloak malign intent.

None of this bears directly on fraud, or on the prevention of it, but there are good reasons for believing that the ordinary defects of the literature arise from the undue importance now attached to the process of publication, largely because of the way in which the careers of younger scientists and the continued success even of successful laboratories have come to depend on what is sometimes known as 'output', which is another name for the number of research articles published, perhaps weighted by length and by the status of the journals in which they have appeared. Some assessment of this kind is the basis of what is called peer-review in the United States (strictly, not to be confused with the process by which journals base their decisions on what to publish on the opinions of external referees). As the economists would say, the research community has externalized the problem of making judgements about the qualities of its own members by brooding on bibliographies. Everybody's interest is to find a less slovenly way of making intellectual judgements about people and institutions. The recipe implicit in the Stewart and Feder argument, that zealous suspicion of everybody within sight is the way to ensure the integrity of the scientific literature, is of course a recipe for disaster, a road to general mistrust, a licence for every would-be whistle-blower and a means by which the literature would be made yet more solemn. Decoupling the literature from promotion prospects would be a better goal. □

AIDS campaign gets off to a sticky start

• Controversial advice to blood donors
• British troops advised to take AIDS tests

London

As part of an unprecedented £20 million health education campaign, the 20 million homes in the United Kingdom will receive a leaflet through the post, within the next three weeks, outlining the dangers of AIDS (acquired immune deficiency syndrome) and how to avoid its spread.

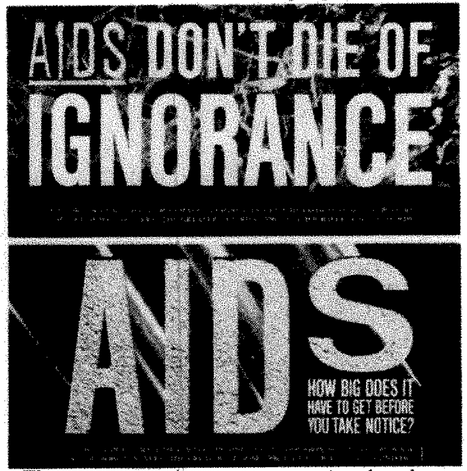
At the same time, there will be a stark television campaign conducted on both the commercial channels and those of the publicly funded networks of the British Broadcasting Corporation, drawing attention to the leaflets and their message "Don't die of ignorance". A special commercial has been made for cinema. The campaign illustrates the growing political pressure on the government from a medical profession that has become increasingly concerned about widespread ignorance of the disease and its frustration in being unable to find a cure.

That frustration was to provide acute embarrassment two days before the launch of the national education campaign. A statement by the British Medical Association (BMA) that those who had had more than one sexual partner in the past four years should refrain from donating blood — a voluntary act without financial reward in this country — caused much confusion and provoked hostility, principally from those trying to build up the resources of the national blood bank.

In response, the health officials, through the Department of Health and Social Security, were forced to play down the danger from AIDS on the eve of a campaign designed to do the opposite. A statement by the government's chief medical officer, Sir Donald Acheson, issued in the wake of the blood transfusion row, illustrated the dilemma.

The original statement read: "In only four of more than three million donations have antibodies to HIV [human immunodeficiency virus] been found in persons who deny being in any of the high-risk groups. This is striking evidence that blood donors who are not in the high risk groups are very rarely infected with HIV." For that reason, men and women who were not in the main risk groups, even those who had more than one sexual partner in recent years, should continue volunteering to give blood, the statement continued. After reflection on this, the BMA withdrew its advice.

The emphasis of the educational campaign was different. Whatever we do, claims Norman Fowler, the Secretary of State for Social Services, it is likely that as many as 4,000 people in the United Kingdom will have died of AIDS in the United Kingdom by the end of the decade. Mr Fowler sees the object of the education campaign as stopping the spread of the virus now and "for everyone to behave in a way which minimizes the chances of their being infected".



AIDS DON'T DIE OF IGNORANCE
AIDS
HOW BIG DOES IT HAVE TO GET BEFORE YOU TAKE NOTICE?

The government's poster campaign has been less cryptic than the advertisements puzzling television viewers.

Despite the apparent contradictions in the two messages, the British medical authorities are seriously worried. The number of AIDS victims is to be published once a month and an interpretation of those statistics provided once a quarter.

One disturbing trend is the proportion of incidence of the disease that appear to be clustered around the London area. Seventy per cent of the 293 deaths in Britain up to the end of last year were reported in the London area. Panic press reports predicted that London would become an 'AIDS capital' while more reasoned articles suggested that the cases had clustered because of the medical facilities available in the capital — the first AIDS ward in Britain is to be opened this week at the Middlesex Hospital in London.

The dangers of the spread of AIDS in sexually active groups has focused attention on the armed forces. The 600 soldiers of the first battalion of the Queen's Own Highlanders have been urged to take tests for AIDS having just returned from an eight-week spell in Kenya, where the disease is thought to be prevalent amongst prostitutes.

Bill Johnstone

Rifkin case thrown out

A US DISTRICT judge has thrown out of court an attempt by social activist Jeremy Rifkin to have declared illegal the US government's plans for regulating biotechnology. Rifkin has argued that the government's "Coordinated Framework" for regulation, published last June, was issued without adequate public participation, and that it lacked a required environmental impact statement. Judge Gerhard Gesell ruled Rifkin's complaint invalid on 22 December because the framework was a "far from successful effort to establish a common set of definitions" and because Rifkin's claim of injury rested on possible future government action.

Rifkin is undeterred by the apparent setback, arguing that the finding that the framework is not a formal "rulemaking" is a victory for his cause. It simply means, he says, that he must in future sue relevant agencies one by one, not *en masse*. ☐

DNA fingerprinting OK

DNA fingerprinting as a way to avoid or settle disputes over family relationships is given guarded approval by the UK Immigration Advisory Service in its annual report. The government-supported voluntary organization outlines the safeguards that it would like to see built into the application of the test to immigration disputes. Testing should not be compulsory, it says, but it would in most cases avoid the problems of trying to establish relationships by interviews. Blood samples would be collected overseas and sent for comparison with samples from relatives in Britain. ☐

Space centre waiting

BRITAIN has no cash to award new development space contracts because of the government's reaction to a 15-year space plan, completed at the end of last summer by the British National Space Centre (BNSC), is more than two months overdue. The delay is causing considerable embarrassment at BNSC, which was formed about 15 months ago to co-ordinate the British space effort. BNSC officials need approval soon if Britain is to participate in international projects such as the European space plane Hermes.

BNSC hopes to be able to negotiate its contributions to the European projects at an ESA meeting in the spring, when preliminary discussions will lay the foundations for a full ministerial meeting in June. France has already announced increased support for space, bringing its total expenditure on space to £700 million — nearly seven times that of Britain. ☐

Correction

THE Reagan administration's proposed budget for the fiscal year beginning next October is $1.024 million million (*Nature* 325, 96; 1987). ☐

US courts and legislatures face implications of surrogacy

Washington

THE volatile issue of surrogate parents is once again bubbling. A test case in a New Jersey court will determine whether a contract requiring a surrogate mother to give up a child to the infant's genetic father and his wife is enforceable. Meanwhile, in New York state, a legislative committee is recommending drafting legislation setting up guidelines to regulate surrogacy, something no other state has yet done.

The New Jersey case arises from William and Elizabeth Stern's attempt to have a child through surrogacy because of Elizabeth Stern's medical condition, which makes child-bearing dangerous. Through the Infertility Center of New York, they contracted with Mary Beth Whitehead, a New Jersey housewife, for her to be artificially inseminated with William Stern's sperm. After being delivered of a baby girl, Mrs Whitehead initially gave her to the Sterns, but later decided she wanted to keep the baby. A court has given the Sterns temporary custody while deciding whether the contract between Mrs Whitehead and the Sterns can be enforced.

The New Jersey case embodies just one of the problems plaguing surrogacy. In the Stern–Whitehead case, both parties would like to keep the baby. But critics ask what would happen if the infant were deformed, when neither party might wish to keep it. Or what if the couple seeking the child simply changed their minds during the pregnancy?

US courts have already made some headway on the difficult issue of the rights of the genetic and the nurturing parents. There is a long-standing legal tradition that the woman who delivers the child is its mother. But H. Tristram Engelhardt, who deals with medical ethics at Baylor College of Medicine in Houston, emphasizes the distinction between a surrogate mother who provides the ovum and one who does not. Engelhardt says it is possible to consider the woman who 'lends' her uterus to nurture a fertilized egg as a temporary foster mother. According to Noel Keane, director of the Infertility Center of New York, a Michigan court has already recognized that the people providing the genetic material are a child's parents, regardless of who is actually delivered of the child.

But this genetic basis of determining parenthood has been challenged. Barbara Katz Rothman, a sociologist at Baruch College in New York, argues that the woman with the large belly and the swollen ankles is the mother, regardless of where the sperm and egg came from. She is concerned that, in the debate on surrogacy, the importance of a woman's nurturing role during pregnancy is being overlooked.

Critics of surrogacy are also concerned about the commercial aspects of the procedure. They regard the payment of a fee to a surrogate parent as tantamount to selling babies. Infertile couples have been willing to pay large sums in order to have children. Typically, a surrogate mother will receive a $10,000 fee; medical and legal costs and payments to intermediaries can put the total cost as high as $30,000.

Angela Holder, professor of clinical paediatrics and law at Yale School of Medicine in New Haven believes surrogate arrangements worked out between close family members or friends, where no money changes hands, are justifiable.

The proposals issued earlier this month in New York, by the state legislature's Senate Judiciary Committee, would give legal sanction to surrogacy and also regulate costs. The committee's report stresses the importance of the informed consent of all parties through independent legal representation. The report urges that courts review all fees involved, using a standard of 'just and reasonable compensation' and that, after successful conception from artificial insemination with the intended father's semen, the surrogacy contract should be binding. The child would be deemed the legitimate natural child of the biological father and his wife. Legislation containing these recommendations will probably be introduced this month.

New York is not the first state to consider laws on surrogacy, but although five states have considered legislation, no laws have yet been adopted. In any case, legislation is unlikely to end the controversy. Lisa Newton, a philosopher at Fairfield University, believes legislation will simply create more difficulties. She believes that legal principles to deal with surrogacy do not exist, and that thoughtful legal decisions to provide precedents for future cases are now needed.

Many countries are wrestling with surrogacy issues. In Britain, the Warnock Report in 1985 urged that surrogacy for profit should be banned, and there is now legislation to that effect. In West Germany, a government commission is expected this week to set guidelines for surrogacy; last September, a conference of lawyers decided that surrogacy did not violate human dignity if the gestational mother provided her own ovum. In France, a presidential panel has been holding debates on the issue, and the Council of Europe has been hearing arguments on a set of provisional principles concerning human artificial procreation. **Joseph Palca**

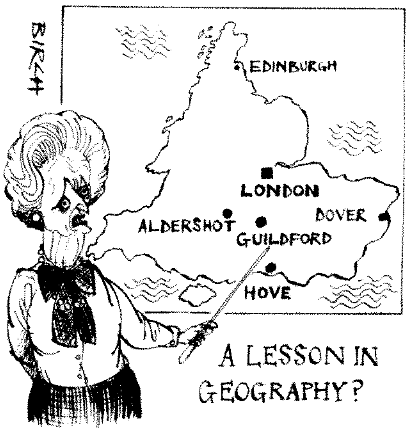
Official: UK's industry in a deep decline

London

A MAJOR political row erupted last week with the publication of the latest employment statistics which showed that Britain's manufacturing industry is in deep decline and is continuing to shed jobs. The statistics, prepared by the Department of Employment and based on its 1984 Census of Employment, showed that the job losses were largely concentrated in the traditional manufacturing areas, mostly outside the affluent South-East.

Two million manufacturing jobs are estimated to have been lost since 1979, with Scotland, Wales, Yorkshire, Humberside and the West Midlands bearing most of the burden.

The political criticisms were heaped on the government in the wake of the publication of the statistics that were hailed as proof that Britain has not properly invested in research and development and is increasingly becoming an assembly shop for foreign manufactured goods. Certainly the Cabinet Office, which is currently attempting to devise a new policy for British science, tailored by an influential secretariat, is highly critical of the amount of money available for development. The research monies are sufficient it claims, but that available to exploit science is inadequate.



The conclusions of the Department of Employment from the statistics indicate that there will be little relief in the manufacturing sector in the immediate future. The latest figures on employment in manufacturing, says the department, show a decrease of 10,000 per month in the three months ending October. Although estimates have fluctuated from month to month, the average rate of decrease so far this year has been 14,000 per month. These revised estimates, the officials claim, "show a greater decrease in manufacturing employment than was previously thought". **Bill Johnstone**

Abortion-inducing drug alarms the right-to-life lobby

Washington

A NEW steroidal compound that may be used for treating cancer, glaucoma and even AIDS (acquired immune deficiency syndrome) is nonetheless at the centre of a political storm because of its most promising property: the ability to terminate early pregnancy with a single, 600-mg dose. The drug, produced by the French pharmaceutical company Roussel-UCLAF, is most commonly referred to by its original code name, RU 486. But opponents of the testing of RU 486 have called it the 'death pill'; they are asking that it should never be made available in the United States.

RU 486 is an antiprogestin that is thought to compete with progesterone at the receptor site. The antihormone–receptor complex cannot trigger the actions normally provoked by the hormone. During pregnancy, the antiprogesterone increases contractility of the uterine muscles, an effect enhanced by prostaglandins. Administration of RU 486 will induce menses in woman who are not pregnant, and abortions — in most cases — in women who are.

RU 486 is also an antiglucocorticosteroid, which makes it useful in treating certain forms of Cushing's syndrome, a disease characterized by increased adrenocortical activity. Similarly, RU 486 may be used to treat other conditions related to excess corticosteroid production, such as lymphomas and glaucoma. By blocking cortisol activity at the receptor level, RU 486 can also interrupt the normal negative feedback control mechanism, stimulating the hypothalamic-pituitary-adrenal axis. This activation can enhance the effect of anticancer drugs, and alter the immune response, potentially relevant to treating AIDS patients.

Approximately 1,500 women around the world have now been given RU 486 to terminate pregnancy. The World Health Organization is conducting a multicentre trial in eight countries (China, India, Hungary, Sweden, Singapore, Vietnam, Italy and Scotland) using a combination of RU 486 and prostaglandins in tandem to induce abortions. The Population Council has also organized studies of RU 486's effectiveness as an abortion inducer both in the United States and overseas. Roussel-UCLAF has conducted its own clinical trials in France. In one such trial, recently reported, RU 486 successfully terminated pregnancy in 85 of 100 women treated within 10 days of their first missed period.

Supporters of the right-to-life movement in the United States are alarmed by the implications of RU 486, which they fear will make abortions easier to arrange, and at the same time diminish a woman's sense that she is taking the life of her unborn child. A group of 6 conservative senators wrote to Secretary of Health and Human Services Otis Bowen last February, asking him to confirm that federal funds were not being used to investigate RU 486 as a potential agent for abortion.

In April, Bowen responded with a list of all projects using RU 486 sponsored by the National Institutes of Health (NIH). NIH have been scrupulously careful about not using their facilities in any way that might be considered related to abortion. Federal law prohibits federal money from being spent on abortion research. Tests on human subjects are all carried out before implantation of the fertilized egg.

Etienne-Emile Baulieu of the Université Paris-Sud, who has developed RU 486, would prefer that the drug should be called a contragestive agent rather than an abortifacient, on the grounds that it interferes with the establishment of pregnancy. But he admits that the political antecedents of the drug may impede its introduction in the United States, where liability issues could also price the drug out of a potentially enormous market.

There are other than social problems for RU 486. The drug has a relatively long half-life — 20 hours — allowing it to remain in the body longer than would optimally be desired. Heavy bleeding follows treatment in some women, which means that taking the drug without medical supervision could prove dangerous.

Despite these problems, other companies are interested in the potential of RU 486. Schering AG in West Germany has developed a similar compound now in clinical trials. And Roussel-UCLAF plans to submit licence applications for RU 486 this summer in France and Sweden.

Joseph Palca

New Australian law on embryos still confuses researchers

Sydney

WHAT is an embryo? The answer could provide a solution to some of the issues raised by the Infertility Medical Procedures Act which came into effect last August in the state of Victoria, Australia. The threat of four-year gaol terms contained in the act brought *in vitro* fertilization (IVF) research in Victoria to a complete halt and prompted Dr Alan Trounson, the leader of Monash University's world-renowned IVF research team, to issue an ultimatum that he and his team would go overseas within six months if they were not allowed to continue research. Three months have now passed (see *Nature* **232**, 748; 1986).

The legislation forbids the destruction of embryos but it never actually defines an embryo and at what point a fertilized egg becomes an embryo, or even what fertilization is. According to Professor Carl Wood, chairman of the department of obstetrics and gynaecology at Monash University and head clinician of the Monash/Queen Victoria Hospital IVF programme, all efforts towards a resolution of the researchers' crisis have been stalled by the notion that an embryo comes into being the moment the sperm head passes into the shell of an egg. Although this is an essential step, there are others that might more logically mark the beginning of an embryo's existence, such as 20 hours after fertilization when the male and female genetic material fuse together or 14 days which mark the first appearance of cells that will have a lineage to the fetus and eventually to the new human. Before the 14th day, all the cells that grow from the fertilized egg go to make up the support systems for the fetus such as the fetal sac and the placenta.

The Standing Review and Advisory Committee on Infertility chaired by Professor Louis Waller has power to adjudicate on the interpretation of the legislation. A request from the Monash scientists for clarification of terms such as embryo and fertilization used in the legislation, as well as what constitutes an abnormal embryo and when such can be discarded, is now before the Waller committee. Adoption of a 20-hour limit for embryos should clear the way for approval of two proposed experiments before the Waller committee. This would be sufficient to ensure that the Monash team stays.

One experiment promises the development of a new method for the treatment of male infertility, fertilization by the microinjection of single sperm, and the other is on the gamete freezing method which involves the freezing and thawing of egg cells. Both experiments involve the fertilization of eggs without implantation — an illegal act while fertilized eggs are classified as embryos.

The Monash team would like to see the adoption of the term 'pre-embryo' for the initial 14-day period as used in Britain; this might eventually lead to a broader range of permissible experimentation.

There is still no indication from the Waller Committee on when it will make its rulings. Trounson sees some irony in the situation. If such a law had been in force six years ago, thousands of families would still be childless.

Charles Morgan

USA, Australia and China collaborate over Pacific Ocean

San Francisco

A RESEARCH programme deemed to be one of the biggest and most sophisticated studies ever assembled to probe the dynamics of storm systems and their influence on upper atmosphere chemistry has just been launched.

The world's highest, coldest thunderstorms, which billow into the stratosphere every January and February above the tropical Arafura Sea north of Australia and the neighbouring Indonesian archipelago, may be the key to a vexing meteorological problem: how gases move between the upper and lower atmosphere.

A US National Aeronautics and Space Administration (NASA) ER-2 jet at an altitude of 20 km will test a novel hypothesis proposed about five years ago that these tropical storms in this restricted portion of the globe throw large volumes of lower atmosphere to higher levels and simultaneously preserve the extraordinarily low humidity of the lower stratosphere. The aircraft test is part of a US$10 million NASA project called Stratosphere–Troposphere Exchange Project (STEP). If the theory is supported, the warm seas northeast of Australia may contain the prime meteorological channel through which the bulk of atmospheric contaminants, including industrial discharges such as ozone-destroying fluorocarbon products, reach the stratosphere. Reginald Newell, professor of meteorology at Massachusetts Institute of Technology and an adviser to the programme, has called the area a "stratospheric fountain".

The single-engined ER-2, a research version of the US Air Force TR-1 high-altitude reconnaissance jet derived in turn from the famed Lockheed U-2 spy planes, left the NASA Ames Research Center in California on 8 January on a flight to Darwin via Hawaii and Guam. Its 1400-kg package of 16 instruments in the fuselage and two under-wing pods is the heaviest science payload the aircraft has ever carried.

Waiting at an Australian Air Force Base near Darwin to begin a month-long assault on large tropical storm buildups is a multinational research team for STEP. The project, five years in the making, is coordinated with two other studies, the Australian Monsoon Experiment (AMEX) and the Equatorial Mesoscale Experiment (EMEX). Participating agencies include NASA, the US National Oceanic and Atmospheric Administration (NOAA), the Australian Bureau of Meteorology, and several university-based groups. In addition, the crew of a research vessel from the People's Republic of China will help, launching radiosonde balloons and carrying a US-supplied weather radar to track storm systems for the combined effort.

While the sailplane-like ER-2 flies above, and into, the anvil-shaped tops of massive storms at 60,000 feet and above, other American and Australian aircraft will inspect lower altitudes. The measurements from the Chinese ship will augment a network of a dozen radiosonde and six radar sites along northern Australia.

NASA aims to sort out the precise nature of an atmospheric transport system known as the Walker Circulation, a convective cell with predominantly east–west orientation. STEP will examine the western end of the Walker Circulation as it gathers moist, warm low-level air flowing from near the coast of Mexico, lifting it up over the Western Pacific to feed dry, cold winds blowing back to the east.

Conventional wisdom has it that tropical thunderstorms flatten out at 50,000 feet or so. "We've seen them to 60,000 feet here, and radar suggests they go even higher," according to NASA meteorologist, Edwin Danielsen.

It is an issue only direct measurement can settle. The ER-2's pilots will fly above and into the cloud sheets swept outward of thunderstorm tops, giving them their characteristic anvil shapes as stormclouds overshoot points of equilibrium, flatten out, and extend downwind. The high-altitude cloud decks, rapidly losing heat to the dark sky above, and absorbing radiant heat from the sea, should become vigorously convective. As the NASA team sees it, they will become heat engines, pumping tropospheric air up, while condensing water into ice that falls back down.

Ozone, photochemical nitrogen compounds, and cosmogenic isotopes such as beryllium-7 and phosphorus-32 characteristic of the upper stratosphere, will be compared to aerosols, water vapour and water ice, carbon monoxide and radon daughters swept up in the tropopause. Temperature, wind speeds and pressure will all be carefully charted.

The project offers another big bonus. Pending approval of Chilean authorities to use an airfield at the southern tip of South America, NASA and NOAA officials plan to send the plane over Antarctica in August and September. This is when the height of the recently reported annual south polar ozone depletion is expected to occur (*Nature* **300**, 686; 1982). The Antarctic mission may uncover the role of man-made pollutants, delivered to the upper atmosphere through such mechanisms as the western Pacific stratospheric fountain, in piercing the hole in the ozone. **Charles Petit**

Italy's nuclear forum is postponed

London

A CONFERENCE billed to "decide the future" of nuclear power in Italy has been postponed, throwing the Italian nuclear lobby into confusion.

Prime Minister Bettino Craxi's Socialist–Christian Democrat coalition government has equivocated on nuclear energy in public, but privately is said to back the country's recent "energy plan" to build two or three pressurized-water reactors (PWRs, Italy's first). The conference plan was supposed to be the political solution. To help satisfy public opinion, the government was (and in principle still is) to hold an open, three-day parliamentary conference covering all aspects of nuclear power, particularly safety. This was due to take place next week; but it always seemed to be a dangerous balancing act destined to receive a great deal of media attention, but with no clear powers of decision, so the postponement is perhaps unsurprising.

The Chernobyl accident last year caused an uproar in Italy. Although the country was only slightly exposed to fallout from the explosion of the Ukrainian reactor, Italian politicians (who may soon face an election, were careful to listen to public complaints, and one after another of Italy's many political parties pledged itself against nuclear power. By late last year there was a political lobby large enough to pose a serious threat to the country's fledgling nuclear power programme.

Not only are the plans uncertain for new PWRs in Italy, but also for existing reactors and those under construction. Only four per cent of Italian electric power is nuclear-generated. (Italy is dependent upon foreign imports for some 80 per cent of its energy, making it one of the highest import dependences in Europe.) Two boiling-water reactors are in an advanced state of construction at Montalto di Castro (between Rome and Pisa), and work has begun on three PWRs sited east of Turin in the Piedmont, east of Milan in Lombardy, and south of Rome. Allowing for the decommissioning of older reactors, this would take Italy to around 25,000 million kWh per year of nuclear generating capacity by 1995, which would still amount to only 10 per cent of the country's requirements for electricity by that date, according to the Italian nuclear and alternative energy agency ENEA. The strategy, according to the president of ENEA, Professor Umberto Colombo, is to "hold the position" until renewable and fusion energy becomes available, although, he admits, that could be "forty to fifty years away".

Safety will be the main issue at the conference, but the Italian commitment to the fast breeder reactor will also be in question. Italy owns a one-third share in Europe's — and the free world's — largest such reactor, the 1,300 MW Superphénix (at Creys-Malville on the French Rhône river), and is also party to a Europe-wide agreement on fast reactor research and development. But the Italian Communist Party (a party of Eurocommunists who distance themselves from Moscow, unlike their French equivalents) has set itself firmly against fast reactors. The Communists are by far the strongest of Italy's left-wing groups, and Colombo is "sorry" that Italian withdrawal from fast-breeder research now seems "likely", although continued participation in Superphénix is ensured (for the electricity it provides Italy). The most immediate impact of withdrawal from research would be the end of the so-called PEC 120 MW (thermal power) experimental fast breeder, designed to study fuel safety, which is now 70 per cent complete on a site midway between Bologna and Florence.

Craxi's conference to "decide" on even this small amount of nuclear power and nuclear research has already begun in one sense: the industry ministry in Rome has sent questionnaires to 150 environmental groups, nuclear power and other institutions, asking for their forecasts of future electricity demand and supply in Italy, their estimates of environmental damage and risk, and for proposals for an institutional framework for managing Italian energy and any accidents that might arise.

The conference proper will consist of analysis of the replies, reports by invited experts from Italy and the rest of the world and most critically a closing assessment by the government.

Then, an interparliamentary commission will attempt to reach conclusions, but as Colombo predicts, the short conference is likely to leave a very divided field. An assessment of likely votes is as likely to sway politicians as an assessment of technical arguments, and for the Italian nuclear power lobby a public "*bella figura*" at the conference must be essential.

Meanwhile, however, the spring date approaches when Craxi (a socialist) has promised to hand over the premiership to his Christian Democrat colleagues. He has refused to do this once before, and many think the same thing may happen again, this time precipitating a general election. That could mean Italy's energy future being decided in the white heat of Italian politics. **Robert Walgate**

Landsat remote sensing project short of funds for satellites

Washington

As a result of a delay in 1987 budget negotiations between Congress and the National Oceanic and Atmospheric Administration (NOAA), work on the two newest Land Remote Sensing Satellites (Landsats 6 and 7) has ground to a halt.

The Earth Observation Satellite Company EOSAT, dependent upon a NOAA subsidy to construct the new satellites, has been forced to terminate Landsat development subcontracts. These contracts were held by the Santa Barbara Research Center of Hughes Aircraft Company and General Electric's RCA Astro-Space Division, among others. Termination of the subcontracts will mean layoffs of up to approximately 200 scientists and technicians for each major contractor, and the loss of 17 marketing support personnel for EOSAT. Although the two currently operating Landsats 4 and 5 will not be immediately affected, a continued lack of funds could drive EOSAT into a worse predicament.

For the past five years, NOAA has been a favourite target of Reagan administration budget cuts, struggling each year to get funding. EOSAT has been prone to the vagaries of the forces influencing the NOAA budget since it was formed to commercialize the Landsats. In 1985, when EOSAT took over the Landsats, Congress decided that EOSAT would receive $250 million in start-up funds, to carry EOSAT through the deployment of Landsats 6 and 7. So far, each year EOSAT's subsidy has been deleted from the NOAA budget by the Office of Management and Budget (OMB), only to be put back in by Congress.

For 1987, EOSAT was scheduled to receive $87 million, but OMB did not approve NOAA's budget. Congress then approved the appropriation of $27.5 million of new money for EOSAT, but demanded that NOAA provide a detailed plan for the use of the funds. The request for a new plan was prompted by delays in the Shuttle programme since the Challenger accident left the Landsats with no launch vehicle.

The current, unapproved NOAA plan calls for a $209.2 million total subsidy, down from the original $250 million, for the development of only one satellite instead of two. Launch is proposed for 1989 using a Titan 2 missile. This means a budget in 1987 of $62.5 million. EOSAT says it requires a minimum of $69.5 millon in 1987, and is still pushing for plans for two new satellites.

Congress, EOSAT, and NOAA are still trying to reach a compromise. Once an agreement has been settled, however, EOSAT will have to renegotiate the contracts with its subcontractors, a task that could set the project for the new Landsats back by nearly two months. Meanwhile, EOSAT, Landsat and the fate of any projects dependent upon Landsat data are caught in budgetary limbo. **Carol Ezzell**

NASA mosquito watch threatened

Washington

PREDICTING malaria outbreaks from space may be possible using a model being developed by the National Aeronautics and Space Administration (NASA). The model will use remote-sensing images taken by Land Remote Sensing Satellites (Landsats) of malaria-prone regions to forecast when and where outbreaks are likely to occur. But the budgetary problems that have recently befallen the Earth Observation Satellite company (EOSAT), Landsat's owner, may mean that projects like the malaria watch will have trouble getting off the ground.

If money problems can be solved, the first phase in the development of the malaria model will take place this summer in the rice fields of Northern California. A local species of mosquito is closely related to the ones that spread malaria elsewhere, although California's control measures have rendered the state virtually free of the disease. According to project chief Paul Sebesta, researchers at the NASA Ames Research Center will be collecting "ground truth" data to work out the relationship between the satellite images of ground reflectance patterns and the variables that influence the proliferation of malaria-bearing mosquitoes. Such ground variables include surface water distribution, rainfall patterns, drainage, vegetation type and temperature. Once these relationships are established, it will be possible to predict both the extent of the mosquito population and how long it will thrive.

The second field trial of the new technique will begin in 1988 in the southwestern oceanic plain of Mexico, near Tapachula, a site with a large malarial mosquito population. The Mexican location was selected during a workshop between NASA scientists and world health officials held early last year at the Uniformed Services Medical Center in Bethesda, Maryland. Criteria for choosing a site were that it should have only one species of mosquito serving as the malaria vector, be politically stable and easily accessible. Costa Rica, Turkey, Pakistan and China were also considered, and they are likely to top the list for future trials. If successful, the model will be turned over to national and international malaria control authorities. Carol Ezzell

Japan overtakes Soviet Union in research spending league

Tokyo

JAPAN's investment in research and development continues to soar, with industry providing the lion's share, although basic science is still short of funds. Research spending rose more than 12 per cent in the last fiscal year (ending March 1986) to surpass that of the Soviet Union; Japan now ranks second only to the United States, according to figures from the Management and Coordination Agency.

Private industry has dominated recent growth in investment while the share of funding for the universities and basic science continued to lag. But in the life sciences, and in particular genetic engineering, there were large increases in both the public and private sectors.

Spending on research and development

Country	R&D expenditures (Ymillion million)	% of GNP
Japan (1985)	8.89	2.77
United States (1985)	25.95	2.72
Britain* (1983)	2.37	2.18
West Germany (1985)	4.23	2.84
France (1984)	2.58	2.10 (1982)
Soviet Union (1984)	7.69	3.73 (1982)

*The figures for Britain do not include the humanities and social sciences. The equivalent figures for Japan are 8.12 million million yen and 2.53 per cent of GNP.

It is a characteristic feature of Japanese science and technology that research funding is provided largely by industry. As a result, the research is predominantly applied and product-oriented. Although some Western governments might be envious of such a situation, it causes great concern in Japanese government circles. The chronic national debt, which now stands at Y152 million million (nearly $1 million million), has prevented any significant increase in government funding for basic research, and the gap in funding between basic and applied research continues to widen.

Total spending on research and development, including outlays for the humanities and social sciences, reached Y8.8 million million ($58,000 million), 2.8 per cent of Japan's gross national product (GNP). Nearly 80 per cent of this was provided by the private sector which increased its expenditures by 15 per cent over the previous year.

The big spenders in industry were the electronics, chemical and car industries, the electronics companies investing Y1.8 million million. Steel and ceramic industries also increased research funding by more than 25 per cent.

In the energy sector, nuclear power research rose 10 per cent to Y350,000 million. With electric power companies raking in profits as a result of low oil prices and the high yen, and with the government and industry holding ambitious plans for expansion of nuclear power, funding for this type of energy research scheme is bound to increase. Outlays for research on natural sources of energy (such as wind and solar power) on the other hand remained stagnant or decreased.

Space and marine research, much of which is funded by the government, show healthy increases. But research spending in the universities, where much of Japan's basic research is carried out, did not even keep up with inflation. Funding for science (including engineering and agriculture) rose a mere 1.1 per cent to Y1 million million. With more than 120 scientists and engineers in the universities, more than 80 per cent of these funds were swallowed up by salaries alone.

Basic science did receive a boost, but not from the government. Industry has begun to perceive the need for basic research (or at least for the kind that might lead to applications), and privately-funded laboratories for basic research have been sprouting up all over the country (*Nature* **311**, 404; 1984). Industry's contribution to basic science increased by more than 20 per cent in fiscal year 1985 to Y350,000 million, a large proportion of this coming from the chemical and pharmaceutical industries. Nevertheless, the proportion of Japan's total research and development funds allotted to basic science continues to decline. The picture in the universities is not all gloom, though, and funding for the life sciences has increased by leaps and bounds.

Prime Minister Yasuhiro Nakasone's enthusiasm for the life sciences and basic research in this field is destined to give these disciplines a further boost this year if his "Human Frontiers Science Program" gets off the ground. Promoted as Japan's answer to the US Strategic Defense Initiative and Europe's Eureka, the frontiers programme is expected to support basic research into the functions of living organisms (including man), such as mechanisms of the brain, and the nervous and immune systems. But even here Japan has its eye on future applications, such as intelligent robots and artificial muscle.

But it is not clear how the funds for such a major initiative will be raised, although tax reform and privatization of the national railways may help to boost government finances in the long term.

David Swinbanks

Cold spell taking its toll in the Eastern bloc countries

London

THE current cold spell has caused considerable disruption to the Soviet Union's already over-stretched energy supplies. With temperatures the lowest of the post-Stalin era, there has been inevitably an increased demand for electricity. But, even before the cold set in the situation was already serious, with rationing and staggered working hours in force in many of the union republics (see *Nature* **324**, 403; 1986). The Chernobyl disaster, and the shortfall of hydroelectric capacity due to the drought last summer, are being cited as the main causes. But other factors are clearly involved. A politburo meeting last August noted the need to step up the refitting and overhauling of existing power stations, the stock-piling of fuel at power stations, and, in particular, the introduction of fuel and energy-saving measures, including the "prewinter preparation of dwelling houses".

The latter point is significant. Shoddy workmanship in the construction of apartment blocks (a major target of the new policy of openness) and infrequent and inadequate maintenance are responsible for considerable waste of heat, while, according to a spokesman for Moscow city council, many of the new blocks were simply not designed for such cold weather.

But the extra fuel needed is not readily available. Coal trains are snowed in and gales in western Siberia have brought down the exposed power lines that serve the oilfields, halting the extraction of oil.

Elsewhere in Eastern Europe, the situation has been similar, with transport paralysed, massive demands on already overstretched energy supplies. In Hungary, an inter-ministerial coordinating commission has been set up to deal with what is officially termed an "extraordinary situation", and rescue work is being undertaken jointly by Hungarian and Soviet troops. (Normally, except during Warsaw Pact exercises, Soviet army units in Hungary keep an extremely low profile.) Bulgaria, which several weeks ago had already urged stringent energy-saving including fines for excessive use, and Romania, where prolonged power cuts occur regularly every winter, expects considerable difficulties in the next few days.

Vera Rich

Danube water-economy plans stir up environmental opposition

London

Last August, when the USSR Supreme Soviet and Central Committee of the Communist Party cancelled the 'project of the century' — the diversion southwards of the north-flowing rivers of Russia and Siberia — the decision gave impetus to the growing campaign for the cancellation of another, less-publicized water-management project, the Danube–Dnieper irrigation canal. The canal, together with auxiliary works including two hydroelectric stations on the lower Dnieper, is known as VHK, the Ukrainian acronym for 'water-economy complex'. But there are growing fears among Ukrainian scientists that VHK may prove a false economy.

Addressing the Soviet Academy of Sciences recently, the president of the Ukranian academy, Academician Borys Paton, claimed that preliminary estimates put the construction costs at more than 30,000 million rubles and that the canal would also create "negative ecological consequences" including the cutting off of estuaries from the sea, the need to install expensive and sophisticated technology to purify the waters of the Danube, the flooding of vast tracts of agricultural land, and the problem of rendering harmless 5 or 6 km^3 (presumably annually) of highly mineralized drainage run-off. An expert commission of the USSR State Planning Commission has demanded a revision of the plans, which have not been formally ratified. But, Paton said, "most regrettably, as happens in our country", the preliminary work has already started.

The main purpose of VHK is to develop the agricultural potential of southern Ukraine and Moldavia. Paton himself is highly critical of current Soviet investment policy in land improvement, which, he told a special session of the Ukrainian academy last year, spends a disproportionate amount on irrigation, to the detriment of anti-erosion and anti-acidification work, desalination and the like.

The use of Danube water for irrigation, in the opinion of the anti-VHK lobby, could cause considerable damage both to agriculture and the river-estuary coastal ecosystem. Ironically, the Kiliya channel, the northernmost arm of the Danube delta, from which the proposed canal would presumably start, is now being developed as a nature reserve of unique ecological significance.

Although the Danube is an international waterway, there is at present no international body responsible for water quality. The Danube Commission, established in 1948 by the Danube Convention which the Ukrainian SSR signed as a separate state (the Moldavian SSR was represented by the Central Soviet government) is concerned only with problems of navigation, and thus only with pollution resulting from ships. There is, so far, no international body responsible for land-based pollution, although much Central European industry is situated along the Danube. In December 1985, a meeting of representatives of the riparian countries was convened in Bucharest to discuss a unified environmental strategy for the river, but this produced little more than a preliminary statement of intent. The opponents of VHK say that the scheme could ruin the "recreational natural resources" of the Ukrainian republic, in which there has been considerable investment in recent years, while, according to Professor B.P. Zahoruyko, rector of the Odessa Maritime Fleet Engineers' Institute, the project could cause "no little harm" to river transport and fisheries. Zahoruyko is particularly alarmed at the proposal to isolate the Dniester estuary and the Belgorod–Dnestrovskii harbour from the Black Sea by a dam.

Whether the protests of the Ukrainian scientists will be as effective as those of the Russians may eventually depend on what factors really decided the cancellation of the north–south diversion. According to Academician Aleksandr Yanshin, a vice-president of the Soviet Academy of Sciences, the decision was made on strict scientific criteria; suggestions that public concern over the environmental consequences played a significant part are "a long way from the truth". Less official conversations with Soviet academicians, including ecologists, suggest that protests from the representatives of the humanities within the academy and from the group of Russian writers known collectively as the the 'villagers' may have helped to sharpen the issues for the experts who took the final decision.

In Ukraine, however, the writers have been less concerned with environmental issues, and so were soundly scolded during the Congress of Ukrainian Writers last summer by the first secretary of the board of the Ukrainian Writers' Union, Pavlo Zahrebelnyy. They should emulate the "boldness" of their Russian colleagues, he urged, and speak up against the "senseless planning" that is inflicting such harm on the environment. If the Ukrainian writers and representatives of the humanities fail to respond to Zahrebelnyy's call, then the ultimate decision on the Danube–Dnieper VHK may well indicate what part public pressure from concerned individuals really played in the cancellation of the north–south diversion. **Vera Rich**

Biotechnology firm turning the corner?

London

Celltech, Britain's only major biotechnology company, together with its two associated companies, threatens to show its first profit in the coming financial year. Although the dominant patent position of Genentech has forced Celltech to stop its development of tumour necrosis factor as a human thereapeutic product, the company is optimistic about gaining a share of the market for tissue plasminogen activator, as it believes that the patent position outside the United States is still open.

Reporting a turnover of £7.6 million with a loss of £0.7 million for the financial year that ended in September 1986, Celltech emphasized that it remains the world leader in bulk production of monoclonal antibodies, which account for most of the £5 million product sales of Celltech, Boots-Celltech and Apcel last year. Hopes run high for sales of a monoclonal-antibody-based test of ovulation. Measuring the ratio of two urinary metabolites of oestrogen and progesterone, the test is expected to become available in infertility clinics within months. But, in collaboration with London International, Boots-Celltech hopes to develop a dipstick-like version of the test to be sold over the counter as an anti-conception aid.

Celltech's chief executive, Gerard Fairtlough, placed particular emphasis last week on two relatively new projects. One is the development of the tissue inhibitor of metalloproteinases as a therapeutic substance. The inhibitor is part of the natural mechanism by which the turnover of connective tissues is controlled and is expected to be useful in the therapy of rheumatoid arthritis and other connective tissue disorders.

The other highlighted project combines Celltech's recombinant DNA and monoclonal antibody expertise. Financed by American Cynamid to the tune of £5 million over two years, it is aimed at cancer detection and treatment with genetically tailored antibodies to tumours, complexed to toxins, drugs or radionuclides.

In a report on Celltech released this month, stockbrokers Wood Mackenzie place the company within the top ten in the world in terms of revenues and describe it as a "robust competitor". Celltech is likely to develop very rapidly over the next three years, says the report, and should be in a position to offer its shares to the public "in 1987 or 1988"; Gerard Fairtlough concurs, adding that any decision to go public will depend on the state of the market and the needs of the company and its shareholders. **Peter Newmark**

Gene manipulation in Britain

SIR—Joseph Palca's article "Living outside regulation" (*Nature* **324**, 202; 1986) reports that a genetically manipulated yeast developed in the United States may be tested in England at least in part to avoid the US regulatory process. The article might be interpreted by some readers as implying that in England regulatory requirements for this type of work are more relaxed than in the United States.

Although I am not in a position to make direct comparison between regulatory requirements in the United States and Britain, I would like to make the point that in Britain the safety issues raised by genetic manipulation have traditionally been handled in a balanced and flexible way as exemplified by the framework for notification, risk assessment and laboratory containment established by the former Genetic Manipulation Advisory Group (GMAG) and the Health and Safety Executive (HSE) in the 1970s.

The Advisory Committee on Genetic Manipulation (ACGM), HSE and other involved government agencies intend to continue this tradition as genetic manipulation finds increasing application outside the laboratory.

Existing requirements in this area are for those proposing to carry out large-scale fermentation of recombinant DNA organisms to notify HSE in advance according to guidelines issued by GMAG in 1979 (revised 1982). Each proposal is then considered on a case-by-case basis by HSE and ACGM. Also, revised and more detailed guidelines on the risk assessment and notification of this type of work are under preparation. In common with genetic manipulation laboratories, large-scale sites are subject to inspection under the Health and Safety at Work etc. Act 1974 by HSE's specialist microbiology inspectors who are also available for advice.

For the environmental release of recombinant organisms, guidelines were issued in 1986 calling for prior notification and setting down risk assessment factors for such work. Notifications are considered by ACGM, its Planned Release Sub-Committee, HSE and other relevant government agencies (the Department of the Environment, the Ministry of Agriculture, Fisheries and Food (MAFF), the Department of Health and Social Security and the Nature Conservancy Council) in advance of any release.

Also the Health and Safety (Genetic Manipulation) Regulations 1978 are under review and this review is expected to take full account of the growing importance of large-scale fermentation and release into the environment. For genetically manipulated organisms as pesticides, the requirements of the Control of Pesticides Regulations 1986 are relevant. The Food Act 1984 places a duty on the processor/manufacturer/retailer to ensure that food offered for sale for human consumption is safe. There is also a notification scheme for novel foods operated by MAFF in the United Kingdom that would apply to the use of free-living microorganisms. Data on foods under this scheme are evaluated by the Advisory Committee on Irradiated and Novel Foods.

It is important that the oversight of genetic manipulation by government strikes the right balance between avoiding undue restraint and ensuring that appropriate consideration is given to potential concerns. On the international front it is to be hoped that the recently published OECD report *Recombinant DNA Safety Considerations* will do much to harmonize national regulatory positions. Meanwhile, reports of regulatory difficulties in one country do not automatically mean that others have the balance wrong.

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An open question?

SIR—Michael Wagner (*Nature* **323**, 664; 1986) implies that a sincerely held belief has the same status as an informed opinion and cites Feyerabend[1] in opposition to Scriven while neglecting to mention that Feyerabend is a 'philosopher' who therein contends that there are different paradigms of rationality and reliance on faith healers is justified because our own paradigm of scientific rationality is founded on irrational elements. Elsewhere[2], Feyerabend maintains that science without sensory experience is possible by means of telepathic communication with a somehow pre-existing, pre-programmed computer. Such is the present state of philosophy.

In countering Marks' claim that there are no theories of paranormal investigation Wagner asserts that "there are, of course, philosophically cautious theories of the paranormal and different models of objective transcendence". One reference given to justify this assertion accepts reincarnation as a fact at the outset and goes on to say that we interact with other realities via higher bodies centred around our physical body, and furthermore: "The hierarchy of realities is topped by the 'absolute'. The absolute is the basis of all realities. In its nonmanifest form, it is potential, intelligent energy. When rippled or modulated, it becomes the basis of our tangible physical matter and individual matter"[3]. This model of philosophical caution provides the considerations that render Marks' "philosophical outlooks" somewhat "problematical".

Wagner admits that there are no scientific theories of paranormal investigation, but only because it "has as yet no paradigm (in the Kuhnian sense)". It is "not yet a mature science" being "in the pre-paradigm stage" and anyhow many would reject the "assumption that scientific thinking is necessarily analytical thinking". The desirability of scientific, analytical thought is thus "an open question".

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Preventing feticide

SIR—In a recent News article (*Nature* **324**, 202; 1986), Radhakrishna Rao reports on the proposed government legislation to restrict the use of antenatal sex determination in India because of the growing problem of female feticide, but the problem may not be restricted to India. For some time now, we in the West Midlands have been aware of the problem of selective termination of chromosomally normal fetuses following amniocentesis for a legitimate purpose, for example to screen for Down's syndrome. It has been our practice (in common with most cytogenetic laboratories in the United Kingdom) to report the karyotypes of individual cases in full, including chromosomal sex. In order to prevent the possible abuse of a costly diagnostic procedure, we have, however, now decided to withhold sex from our reports, unless it has some direct clinical relevance (for example sex-linked disease prediction).

From 1 January 1987, it will be possible for an individual consultant to learn the fetal sex upon special request to the laboratory, but no longer will the chromosomal sex be routinely reported upon and freely displayed in the obstetric notes.

Perhaps the Indian government could make it similarly difficult to learn the chromosomal sex from written laboratory reports, rather than legislate against the useful diagnostic practice of antenatal chromosome analysis *per se*?

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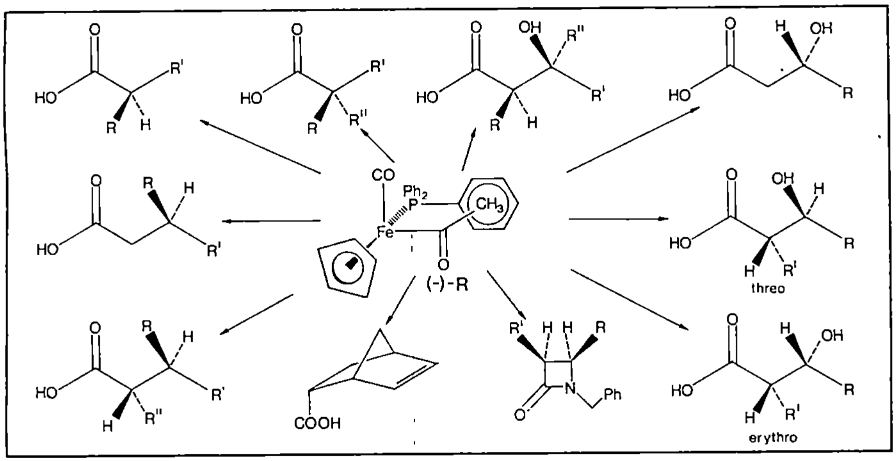

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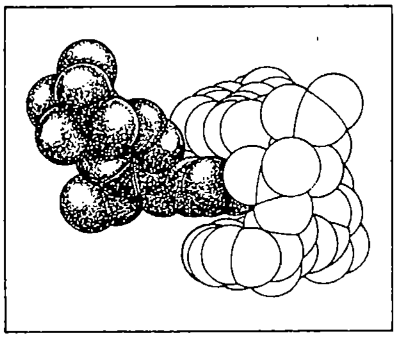

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The domino effect explained

Diplomats should be glad to know that the bar-room game of letting dominoes knock each other over has a theoretical foundation at last. But there is much to be done before the theory will be satisfactory.

Since the flowering of the Cold War, diplomats everywhere have known what is meant by the domino effect; one country falls under pressure from an aggressor and, in doing so, knocks over its nearest neighbour, which knocks over the next, and so on. Not all diplomats may know that the phrase comes from the childish practice, a by-product of the game called dominoes, which is evidently itself a mediaeval poor man's card game, of standing small rectangular tiles on their smallest faces in such a way that each will knock over the next in line. Occasionally, the serious game is buried by the party trick that springs from it, as when television programmes show thousands of carefully prearranged dominoes falling to order.

Reprehensibly, all these exercises appear hitherto to have been based on a strictly empirical foundation. Even the latest attempt to provide a theory of the domino effect, by W.J.Stronge of the Engineering Department at the University of Cambridge (*Proc. R. Soc.* A**409**, 199–208; 1987), is applicable only to the simplest of all possible cases in which the dominoes stand and fall in a straight line.

Although Stronge's account of his work includes some comparison with experiment, nothing is said of when attention at the University of Cambridge will turn to the interesting cases, those when television producers try to persuade falling dominoes to generate a visually recognizable pattern. Attempts to simulate national flags seem to be especially popular, but advertising slogans are a possibility.

So far as it goes, Stronge's analysis is interesting enough. The starting assumption is that there is a one-sided infinity of dominoes standing vertically, with their broadest faces parallel to each other. The geometry of the problem is simplified by assuming that the spacing between consecutive dominoes is a constant, λ; two other parameters matter, the width of the base on which the domino stands, which is really the thickness of the tile, h, and the height of the domino, L, which determines the position at which a falling domino will strike the next.

Stronge's interest is to find the conditions under which an impulse strong enough to fell the first domino will send all the others falling or, not quite the same thing, to tell when and how successive collapses will propagate as a wave along the chain. One way of tackling this problem is to require that the kinetic energy of a falling domino, at the instant it hits the next, should not be less than the kinetic energy of the second when that, in turn, hits its successor. The kinetic energy of a falling domino by the time it hits the next can be calculated from the impulse derived from its predecessor and from the potential energy added by the decreased height of its centre of gravity.

Two high-school complications enter. The collision between one domino and the next will not be perfectly elastic, which means that the impulse acquired by a falling domino will be less that that transferred to the next in line. The usual statement is that the differences before and after impact of the velocities of the falling and struck dominoes, measured perpendicular to the face of the target, are in the ratio of the coefficient of restitution, e, which is a number less than unity unless the collision is elastic. Another complication is that of friction, a thief of energy at each collision as the impacting edge of a falling domino slides across its target.

The upshot of these calculations is that, for each geometrical arrangement of the dominoes, there is a characteristic speed of propagation of catastrophe determined by a characteristic velocity of impact of a falling domino with its successor. Moreover, if the object at the beginning of the line is set moving more quickly than required, extra energy will be dissipated until the characteristic speed is reached. The same is true if there is too little energy to begin with.

Stronge's chief result, a formula relating the characteristic impact velocity in a domino array to its geometrical properties and the dynamical characteristics of the objects, e and the coefficient of friction, μ, will unfortunately not be directly of assistance to those wishing to display the domino effect. The parameters are too deeply buried in the trigonometric functions. But what the inevitable numerical evaluations show is that, except when friction is important, the characteristic impact velocity increases with the distance between the dominoes (which is not surprising, for each then has further to fall before it hits the next).

The sensitivity of the result to the value of the coefficient of restitution, e, arises because the formula for the critical speed takes the form of an algebraic fraction whose denominator is $R^2 - (1 + e^2)$, where R is a quantity involving the coefficient of friction and the geometry of the dominoes and whose value is numerically 2 when friction is literally zero. If e were also unity, meaning that no energy was lost in collisions, the characteristic impact speed would be infinity. That makes sense, for then the potential energy of all the fallen dominoes would be concentrated in that about to fall. No doubt special relativity would have to be invoked.

Unfortunately, Stronge has little to say about the speed with which catastrophe propagates along a line of dominoes. The reasons are understandable: the speed will be determined by μ and by the time that lapses between the point at which a domino is struck by its predecessor and that at which it hits the next in line, a quantity essentially that spent by a compound pendulum on part of a cycle, yielding an elliptic integral. But Stronge does provide three calculations of the speed of a wave of catastrophe in a line of realistic dominoes — giving values ranging between 0.65 and 0.86 m s^{-1} as the spacing between the objects increases.

Quite apart from the restriction of this theory to linear arrays of dominoes, some awkward mechanical problems have also been overlooked. Thus the experiments at Cambridge, in which falling dominoes have been recorded by a cine camera, deny the assumption that each domino is knocked over by a single impact by its falling neighbour. It also seems that frictional losses may be especially important when dominoes are stood close together. Then the calculations depend critically on the assumption that the dominoes stand on a perfectly rough (slip-free) surface, which may not always be the case.

One general conclusion shining through these uncertainties is that, for a specified geometry, there must be a critical spacing below which no impulse, however great, will successfully propagate along the line. Physically, the energy will be absorbed in tilting dominoes so that they simply lean against each other.

It remains to be seen where this first comprehensive attempt at the theory of the falling domino will lead. Plainly there is an urgent need of more realistic calculations. Whether those with practical needs will be able to wait that long is another matter. But Stronge's theory is certain to stimulate a rash of empirical investigation in bar-rooms and other places where these investigations are habitually pursued.

John Maddox

Plant molecular biology

Transgenic cereals by direct injection of DNA into plants

Conrad Lichtenstein

Currently there are two successful approaches that can be used to transform plants genetically, but each method has limitations that are delaying the application of the techniques to certain commercially important crops. On page 274 of this issue[1], A de la Peña, H. Lörz and J. Schell report a significant step in applying a new method to cereals.

The first of the two current methods exploits a natural genetic engineer, *Agrobacterium tumefaciens*. This soil microbe contains a tumour-inducing (Ti) plasmid that transfers a DNA segment (the T-DNA) from the plasmid to the nuclear genome of infected plants (or *in vitro* to plant tissue). The method is restricted to dicotyledenous plants (dicots); monocotyledenous plants (monocots) are usually not susceptible (see my recent News and Views article[2] and refs 3 and 4 for reviews). See last week's issue[5], however, for an exception.

The second method involves direct transfer of DNA to plant protoplasts, prepared by enzymatic digestion of cell walls, for example by chemically stimulated uptake using polyethylene glycol[6] or a high-voltage pulse (electroporation), generating transient 'holes' in the protoplast membrane[7]. This method depends on a tissue culture system that allows regeneration of mature plants from protoplasts. Both methods use dominant selectable markers (for example, kanamycin resistance) to select for the transformed tissue which can then be screened for expression of co-transferred but unselected gene(s).

A major class of crops, the cereals, have so far evaded genetic manipulation. Because they are monocots they are not susceptible to agrobacterial infection and only in rice has it been possible to achieve plant regeneration from protoplasts. In this issue[1], de la Peña and co-workers describe a novel approach to genetic transformation of cereals obviating the need for *in vitro* tissue culture and regeneration. The authors injected DNA encoding kanamycin resistance into the developing floral side-shoots (tillers) of rye plants (*Secale cereale* L.) and demonstrated that some progeny had acquired kanamycin resistance by incorporation of this DNA.

Within the floral tillers are archesporial cells; these give rise to pollen in the developing pollen sac by two meiotic cell divisions. Previous work by de la Peña *et al.*[8] had shown that these cells are permeable to colchicine at a stage 14 days before the first meiotic metaphase; colchicine disrupts meiosis and thus provides an assay for uptake.

This result suggested that such cells might also be able to take up larger molecules such as DNA. Thus, de la Peña *et al.*[1] injected a plasmid encoding kanamycin resistance, and under the control of a promoter known to drive transcription in plants, into the floral tillers of about 100 rye plants. After cross-pollination with other similarly injected plants the seeds were harvested and germinated on medium containing kanamycin. Of about 3,000 seeds so germinated, seven seedlings remained green, indicating resistance to kanamycin — sensitive seedlings bleach and die. Of these seven, further biochemical analyses showed that two had aminoglycoside phosphotransferase activity (encoded by the kanamycin resistance gene). DNA hybridization studies confirmed that this expression results from incorporation of the plasmid DNA. The plasmid DNA integrates in multiple copies, some of which show DNA rearrangements.

The selection method shows a background of kanamycin-resistant, yet untransformed, seedlings and the efficiency of transformation is low (about 0.07 per cent). A remaining question is whether unselected but co-injected DNA will integrate and express alongside the resistance gene at frequencies sufficient to allow detection by screening transformants.

Notwithstanding these provisos, one exciting prospect is that this simple transformation procedure can be applied to other cereals that currently lack transformation systems by DNA injection into similar pre-meiotic germline tissue. □

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Antigen recognition

Class discrimination in the world of immunology

Michael J. Bevan

The upheaval which occurred in the early 1970s with the demonstration that helper and cytotoxic T lymphocytes recognize foreign antigens only in association with self surface glycoproteins encoded by the major histocompatibility complex (MHC) continues to be felt. Although we have grown used to the idea that helper T cells recognize degraded (peptide) forms of soluble protein antigens in the context of class II MHC products, we now have to cope with the realization that class I-restricted cytotoxic T lymphocytes, which are usually specific for cellular antigens such as viral proteins, also recognize degraded forms of the antigen.

It makes biological sense to direct lytic T cells towards the infected cell and to focus helper T cells on soluble antigen in order to increase the production of antibodies that will neutralize toxins or viral particles. But if both subsets recognize peptide fragments of foreign antigen, how is this distinction made?

It now seems that it is the responsibility of the two classes of MHC molecules to distinguish between soluble, endocytosed antigens and cellular antigens. The way this is accomplished, as discussed recently by Germain in a News and Views article[1], may be by the non-random intracellular trafficking of vesicles that contain either class I or class II molecules. Vesicles that contain class II but not class I molecules intersect with the endocytic pathway and may present peptides derived from pinocytosed proteins. Class I-containing vesicles receive the peptide products derived from endogenously synthesized proteins. The latest information on the class I MHC-restricted recognition of integral cell membrane proteins is provided by two papers recently published in *Nature*[2,3].

Previous work on the class I-restricted cytotoxic T-lymphocyte recognition of cells expressing influenza nucleoprotein established that fragments of the protein were recognized[4,5]. But this protein is made on free ribosomes. Thus, these experiments left open the question of whether integral membrane proteins, which are synthesized on membrane-bound ribosomes to be transported through the endoplasmic reticulum and Golgi complex and expressed in native form alongside class I molecules, would also be recognized as peptides by class I-restricted cytotoxic T lymphocytes. The new work of Maryanski *et al.*[3] shows that when mice are immunized against syngeneic tumour cells that have been transfected with the fully functional gene for a human class I protein, cytotoxic T lymphocytes are generated that recognize a fragment of the human antigen in association with self class I molecules. The site on the human protein recognized by some class I-restricted T cells is defined by a stretch of 16 amino acids. The complete human class I antigen is simultaneously expressed at the surface, but this native form seems irrelevant for restricted recognition. The work of Townsend *et al.*[2], although not defining a peptide sequence of influenza haemagglutinin, shows that whether leader-sequence intact haemagglutinin is produced and expressed as an integral cell membrane component or whether leader-sequence deleted haemagglutinin is made in the cytosol and rapidly degraded, the same class I/haemagglutinin determinant is displayed at the surface for recognition. This means that the form of haemagglutinin recognized is denatured and implies strongly that it is a degraded form. The important message from this and other work is that irrespective of whether proteins are normally made in the cytosol or on the endoplasmic reticulum, they all provide degradation products that can associate on the cell surface with class I MHC molecules for the perusal of cytotoxic T lymphocytes.

This pathway of degradation of endogenously synthesized proteins leading to class I presentation seems to be different from that for the degradation of soluble exogenous proteins taken into the cell by endosomes and leading to class II presentation. For example, the latter pathway is sensitive to the drug chloroquine, whereas the former is resistant, and peptides derived from endogenously synthesized haemagglutinin might not interact with the class II pathway[6].

Now that we are confronted with the fact that degradation products of endogenously produced proteins (whether originally destined for the nucleus, cytosol or membranes) can be presented on the cell surface with native class I molecules, we can ask whether class I presentation ever involves protein antigens that come from the exterior of the cell. This is central to understanding the induction phase of a class I-restricted cytotoxic T-lymphocyte response to certain types of viral infections. If the only cell capable of presenting antigen to class I-restricted T cells is the infected cell itself, then sensitization would have to occur peripherally in the case of a virus that did not productively infect cells in the lymphoid organs. This is at odds with an old dogma that immune sensitization occurs centrally, in draining lymph nodes or in the spleen. There is, however, evidence in the transplantation literature suggesting that antigens can be picked up from the periphery and presented in the central lymph nodes. For example, female mice of a given H–2 type can be primed to reject a syngeneic male graft by cells from a male of a different H–2 type — presumably because the male-specific antigen is picked up and presented on host cells. In most cases, however, one does not know if the rejection mechanism is dependent on class I or class II MHC presentation. But from *in vivo* systems, there is clear evidence for such class I antigen presentation. Class I cytotoxic T lymphocytes can be primed to foreign minor antigens or to simian virus 40 T antigen that have been introduced on MHC-different cells[7,8]. Furthermore, minor antigens may be picked up and presented by cells to tolerize class I-restricted T cells[9]. In these cases, the foreign antigens must have been taken up by host cells and presented in association with their self class I molecules.

How can one explain the class I presentation of these exogenously derived cellular antigens? One way is to propose that the cell synthesizing the foreign antigen is also producing and leaking processed peptides that may bind class I molecules on cells of the lymphoid system. That this is feasible is demonstrated by the finding that synthetic peptides can be added externally to cells *in vitro*, thereby creating the cytotoxic T-lymphocyte target antigen[3,4]. But it does not seem to be a likely occurrence *in vivo* where peptides are being flushed from the system, nor does it offer a basis for selecting for class I over class II presentation. Another more plausible way to take cellular antigens that are exogenous and to present them as endogenous, class I-associated antigens is via specialized antigen-presenting cells that phagocytose large cellular debris and shuttle the resulting peptide degradation products to their endogenous class I presenting system. Such a phagocytic cell may or may not express class II molecules.

The important distinction from the class II presentation systems here is that the exogenous antigens come as cell debris and not as soluble material or small particles which can be pinocytosed. Such a

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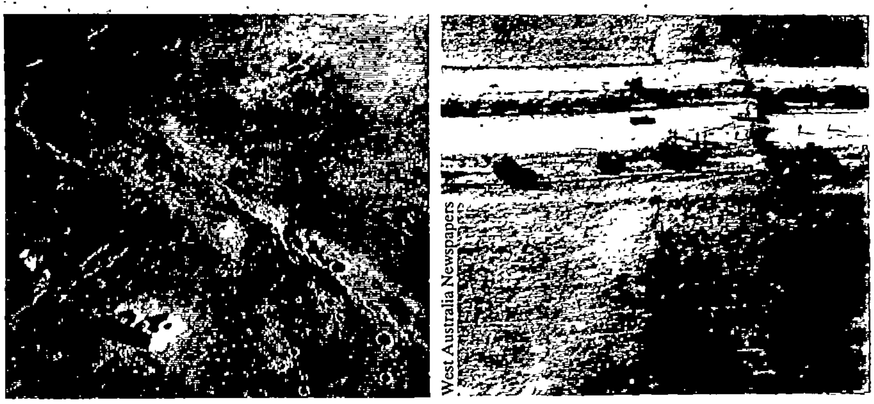
Wrinkle ridges on the Moon and the Earth


West Australia Newspapers

STUDY of the surfaces of Mars, Mercury and the Moon has revealed the existence of wrinkle ridges, complex geomorphic structures variously proposed to have volcanic, tectonic or combined volcanic/tectonic origins. Generally they consist of long linear ridges, with heights measured in metres, that may stretch for hundreds of kilometres. Such a lunar wrinkle ridge is shown (left) in a photograph of the southwestern part of the Mare Imbrium taken from Apollo 15; the field of view (about 370 km across) gives some idea of the scale of these features. Analysis of the ridges to determine their origins has been hampered by the absence of terrestrial data from which to extrapolate. J.B. Plescia and M.P. Golombek have recently investigated structures of Earth that they believe are the analogues of the planetary ridges; their report can be found in *Bulletin of the Geological Society of America* 97, 1289–1299 (1986). A typical terrestrial ridge of total length 37 km is shown (right), which formed after an earthquake at Meckering, Western Australia; the road and vehicles allow scaling. From this and other examples from many widespread localities, the authors argue that the ridges on Earth are the terrestrial equivalents of planetary wrinkle ridges. The major discrepancy is one of scale, in that the examples on Earth are considerably shorter; this is explained in terms of the relatively heterogenous terrestrial stress systems, which effectively limit fault development. Planetary wrinkle ridges, it is suggested, developed following deformation associated with thrust faulting close to the planet surface.

form of presentation would, of course, put the presenting cell at risk of being killed by class I-restricted cytotoxic T lymphocytes but, guarding against this, induced actively cytotoxic cells may move into the blood and lymph rather than stay in the central lymphoid organs. Are there two forms of presentation of exogenous antigen, one the endocytic pathway for soluble antigen operative in all cells but immunologically relevant only in cells which express class II molecules, and another performed only by phagocytic cells? Time will tell. ☐

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Structure determination

Solid-state NMR comes of age

Colin A. Fyfe

HIGH-RESOLUTION nuclear magnetic resonance spectroscopy (NMR) of liquids and solutions has developed into the most widely used analytical technique for the determination of the structures of unknown chemical compounds. It has recently become possible to obtain similar information from solids which, when used together with the information from traditional X-ray diffraction techniques, yields a complete picture of the structure of many materials. Elsewhere in this issue Kirkpatrick and co-workers (*Nature* **325**, 236; 1987) describe work on plagioclase feldspars that represents the state-of-the-art in this area, particularly because the complementary nature of the NMR and diffraction techniques is fully exploited.

In high-resolution solution NMR, signals are observed whose frequencies (chemical shifts) are diagnostic of the local magnetic (chemical) environment and, most importantly, whose relative intensities reflect the numbers of nuclei in the different environments. These two elements are critical in the determination of unknown chemical structures. However, NMR experiments on protons in solid materials yield only broad, featureless absorptions as the direct dipole–dipole interactions between the abundant ^{1}H nuclei are orders of magnitude greater than the chemical shifts from which structural information is obtained.

By working with dilute nuclei such as ^{13}C and ^{29}Si, and using high-power decoupling to remove dipolar interactions from the protons, 'magic-angle' spinning spectroscopy of the sample (Andrew, E.R. *et al. Nature* **182**, 1659; 1959 and Lowe, I.J. *Phys. Rev. Lett.* **22**, 285; 1959) to produce the isotropic chemical shifts and cross-polarization (Pines, A., Gibby, M.G. & Waugh, J.S. *J. chem. Phys.* **56**, 1776; 1972) to enhance the signal-to-noise ratio, J. Schaefer and E. Stejskal of Monsanto were able to obtain spectra of good resolution from completely solid samples (*J. Am. Chem. Soc.* **98**, 1031; 1976). In the case of many inorganic materials such as glasses, zeolites, minerals and clays, the interactions of protons are not important and the experiment is the very simple one of magic-angle spectroscopy alone.

Suddenly, these experiments opened up the possibility of obtaining structural and chemical information on a range of materials and catalysed an upsurge of interest in many aspects of solid-state chemistry. The emphasis in most of these early studies was in extending the techniques and in applying them to different systems. Now, however, it is possible to see in more general terms how the early work fits in with the data provided by the more traditional diffraction investigations of solid systems.

In the case of amorphous materials such as surfaces, polymers and glasses, the NMR data are particularly important because diffraction studies yield extremely limited information as there is no long-range order in amorphous solids. Even in cases where single crystals are available, problems can arise where the structure is dynamic and/or disordered in nature or where individual atoms are difficult to distinguish (such as silicon and aluminium in minerals and synthetic zeolites) and the combination of the two techniques yields a more complete description of the structure. Because the NMR experiments provide the same information (isotropic shifts) in both phases, they can provide a bridge between phases, for example, the structure that exists in a single crystal as determined by X-ray diffraction techniques and the one that exists in solution.

The investigation of zeolites and minerals is a good example of the development of the combined use of NMR and diffraction data. The two techniques are complementary as NMR monitors the local environment (geometry of the atom, nature of the attached groups and local ordering) whereas diffraction data monitor long-range periodicity and lattice networks. Together, the two methods provide a complete description of the structure. Zeolites, open framework aluminosilicate structures, are widely used as catalysts, sorbents and molecular sieves in industry (Breck, D.W. *Zeolite Molecular Sieves* Wiley, New York, 1974). They are microcrystalline in nature and only limited powder diffraction data are available. Because silicon and aluminium are adjacent in the periodic table, they have almost identical scattering factors, which makes it difficult to distinguish between them. In most systems, the atoms are disordered throughout the lattice. This distribution of local environments is exactly the kind of information which NMR can provide. E. Lippmaa *et al.* (*J. Am. Chem. Soc.* **103**, 4992; 1981) showed that the ^{29}Si magic-angle NMR spectra of these systems exhibit to a first approximation five peaks corresponding to the five local environments Si[^{4}Al], Si[^{3}Al,Si], Si[^{2}Al,^{2}Si], Si[Al,^{3}Si] and Si[^{4}Si] describing the average distribution of silicon and aluminium throughout the lattice, and this has been confirmed and extended by others (Fyfe, C.A. *et al. Angew. Chem. Int. Edn* **22**, 259; 1983). There is a constant and additive effect on the shift for each aluminium atom added to the local environment. In addition, for highly siliceous systems where all environments are Si[^{4}Si], signals are observed because of each of the crystallographically inequivalent sites in the unit cell (Fyfe, C.A. *et al. Chem. Lett.* **10**, 1547; 1983), and these shifts can be related to the local geometries of the atoms (see Smith, J.V. *et al. Nature* **303**, 223; 1983 and Thomas, J.M. *et al. Chem. Phys. Lett.* **102**, 158; 1983) and the effect of disorder can be described (Fyfe, C.A. *et al. J. Am. Chem. Soc.* **106**, 4435; 1984).

From these and related studies a clear set of general rules have emerged that can be used in extensions of the techniques to investigations of other aluminosilicate structures, most importantly minerals. The plagioclase feldspars described by Kirkpatrick *et al.* in this issue are the most abundant aluminosilicate minerals of the Earth's crust, but their detailed structures exhibit very complex and subtle variations and are quite imperfectly described, as is the distribution of silicon and aluminium atoms in all phases. The authors used ^{29}Si magic-angle NMR to investigate a series across the solid solution from ordered albite ($NiAlSi_3O_8$) to anorthite ($CaAl_2Si_2O_8$) at the two extrema. In the case of highly ordered albite, it is possible to assign the local environments related to the three peaks in the spectrum as is the case for these in the spectrum of a very special highly ordered anorthite. From these data it is possible to assign the peaks and interpret the distribution of local environments of the intermediate phases and to combine this information with that already available from diffraction data to propose mechanisms for the structural variations of the intermediate phases. Experiments of this type on disordered minerals and ceramics are currently under way in several laboratories. ☐

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Neurobiology

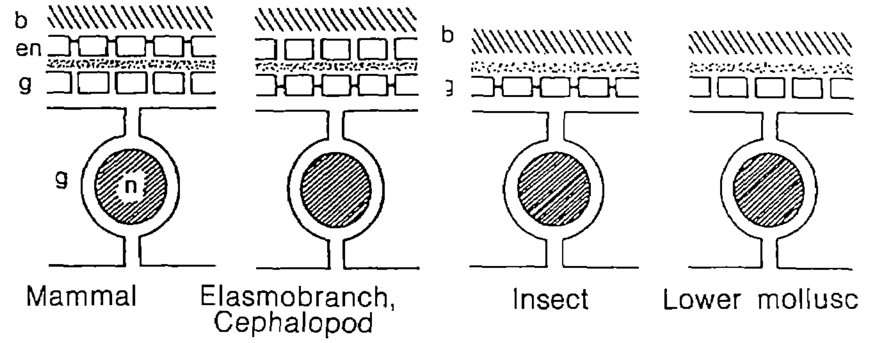
Glia and the blood–brain barrier

N. Joan Abbott

SINCE the classic dye experiments of Ehrlich[1] in 1885 it has been known that many substances injected into the blood have limited entry into the mammalian brain. Rival theories to explain this effect were a restricted extracellular space in the brain, or a barrier at the blood–brain interface. The proposed barrier was also variously attributed to the endothelium, the basement membrane or the end-feet of glial cells (astrocytes), which together form the brain microvessel wall. It was not until 1969, when Brightman and Reese[2] showed that the electron microscope tracer horseradish peroxidase was blocked at the level of endothelial tight junctions when injected into the blood or into the brain, that the debate was resolved. On page 253 of this issue[3], R. C. Janzer and M. C. Raff provide clear evidence that the blood–brain barrier is induced by astrocytes. This result not only provides another function for astrocytes, but also could help to explain the breakdown of the blood–brain barrier that occurs in some pathologies of the brain[4]. However, comparison of the barrier layer in different animal groups suggests that the induction story will not be straightforward.

The role of perivascular glial cells has been tantalizingly difficult to establish. The well-ordered 'pavement' of glial end-feet on the endothelial wall suggested that these cells in some way influenced blood–brain exchange. There were doubts that the endothelial layer could account for the observed rate of bicarbonate transport at the barrier, and transport by the glial end-feet was suggested[5]. Orthogonal assemblies of particles are clustered on the endothelium-facing but not on the lateral membranes of astrocytes, indicating that the cells are polarized[6]. The functions of the assemblies remain obscure, although it is possible they are involved in ion transport. Neurons release potassium ions when they are active. Astrocytes have a high density of potassium channels on their end-feet[7], providing a possible route for the 'siphoning' of potassium away from active neurons, so helping to maintain a stable neural environment.

Even before the location of the mammalian blood–brain barrier was known with certainty, Oldendorf[8] in 1967 proposed that the barrier could be maintained by the influence of astrocytes on endothelial cells. Svendgaard *et al.*[9] provided the first experimental evidence that brain tissue induces blood vessels to form a tight barrier by showing that two specific properties of the blood–brain barrier involving neurotransmitters, an enzymic trapping mechanism for L-DOPA and a barrier for catecholamines, develop in brain fragments transplanted into the anterior chamber of the eye but not in iris fragments grafted into the brain. That is, the donor tissue and not the ingrowing vessels from the host control the vessel wall function. Stewart and Wiley[10] were able to confirm this result in elegant grafting experiments between chick and quail embryos, where they unequivocally demonstrated the origin of vascular elements from host tissue.



Blood–brain interface of different animal groups. Location of barrier layers is shown by bars connecting adjacent cells. b, Blood; en, endothelium; g, glia; n, neuron (modified from ref. 14).

Janzer and Raff's new work clearly shows an inductive effect by astrocytes, both in the rat and the chick, where invading host blood vessels become relatively impermeable to albumin within an exogenous astrocytic mass. The use of primary cultures of identified type 1 astrocytes is a significant advance on earlier work. The next step will be to establish to what extent these induced barriers mimic the normal blood–brain barrier, for instance in impermeability to ions and presence of carrier mechanisms. Monolayers of brain endothelial cells seem to be capable of forming junctions tight to horseradish peroxidase even when cultured without astrocytes, but these junctions are still of relatively low resistance[11].

What is the nature of the inducing signal that Janzer and Raff demonstrate? Is it a diffusible substance, or an effect communicated by cell contact? The presence of tight junctions in blood vessels at the brain surface that lack an astrocytic sheath[12] suggests the inducer is diffusible and is secreted into the interstitial fluid and cerebrospinal fluid of the brain. Is it produced by isolated astrocytes, or by astrocytes only in the presence of endothelial cells? The model systems introduced by Janzer and Raff will be extremely useful for elucidation of the biochemistry of the interaction.

Some intriguing questions remain. Many invertebrate groups have no blood–brain barrier. Insects, cephalopods and some arachnids do have a barrier, but it is glial, not endothelial[13,14] (see figure). What is the inducing signal here, and how is the need for a barrier and its location decided? Even in mammalian brain, the barrier isolating parts of the nervous system from blood is not always at the endothelial layer — for instance it is found instead at the level of ependymal derivatives in the choroid plexus and over neurosecretory zones, and tight junctions between parenchymal glia may contribute to the isolation of neurosecretory regions from the rest of the brain[14]. Are these barriers induced by a different signal, or have the endothelial cells here lost their sensitivity to the inducer? Among vertebrates, one anomalous group, elasmobranch fish, has its blood–brain barrier at the level of perivascular glial cells, although groups presumed close to the ancestral elasmobranch have an endothelial barrier[15]. What happened to the inducing signal or endothelial sensitivity in elasmobranch evolution? It seems certain that the induction of blood–brain barrier properties will turn out to be a complex and fascinating story. ☐

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Biology of communication

First squeaks of speech?

John C. Marshall

THE two hemispheres of the human brain are (roughly) symmetrical at the level of gross anatomy, yet their functional capacities are quite distinct; many domains of visuo-spatial cognition have their neuronal substrate in the right hemisphere, whereas the left is specialized for core aspects of language and speech processing[1]. Although these latter capacities are species-specific properties of humankind, we might nonetheless expect to find, in other animals, some of the underlying biological mechanisms upon which the evolution of language was built[2]. In a study reported on page 249 of this issue[3], G. Ehret, of the University of Konstanz, shows that the perception of communicative calls is lateralized to the left hemisphere in a comparatively lowly species — the house mouse.

A mouse pup that strays too far from the litter will emit ultrasonic calls within a narrow frequency range, and these sounds provoke the experienced mother to retrieve the pup to the nest. The mother is innately pre-wired to attend to ultrasounds, which are categorically centred on the frequency of 50 kHz, but must learn the significance of these 'distress' calls from interaction with her pups.

In Ehret's first experiment, the maternal motivation of primiparous lactating house mice was raised by removing pups from the litter and distributing them along a running board which extended to the right and left of a central nest. After the mother had retrieved the pups and replaced them in the nest, two loudspeakers, each located at a different end of the running board, were switched on. One emitted 50-kHz and the other 20-kHz tone bursts. When tested binaurally, the lactating mice would leave the nest and reliably approach the 50-kHz ultrasound source in preference to the 20-kHz source. This preference persisted when the mothers' left ears were plugged to attenuate the signals by 40 dB, but each sound source was approached equally often when only the right ear was plugged.

100 years ago

A MOST extraordinary snowstorm occurred here today (January 7). In fifty years' experience I have seen nothing like it. It would be impossible to realize the gigantic size of the snowflakes without seeing them. I can only compare them to a fall of oranges, though the diameter of an orange would be small in comparison with thousands of these snowflakes. Snow had been falling with a slight thaw from 10 a.m., the snowflakes being small. Suddenly, at 12h 12m p.m. they became 2½ inches in length; at 12h 14m they had increased to 2¾ inches. At 12h 16m the flakes had increased in size to 3½ inches (and several measured were 4 inches across). Several flakes were sketched before they began to melt, and one of the sketches is sent as an illustration.

From *Nature* **35**, 271; 20 January 1887.

Because, as in man, the auditory system of the mouse has primarily contralateral neuronal pathways from ears to cerebral hemispheres, the result demonstrates that the left hemisphere is specialized for some aspect of the complex task that the mice are here engaged upon. But which aspect? Perhaps the right hemisphere can 'hear' both 50- and 20-kHz tones but not discriminate between them. Perhaps it can so discriminate, but not link the discrimination to the respective sources of the tones. Perhaps the right hemisphere can succeed in both these steps, but does not 'know' that 50 kHz signifies distress (and thus does not 'care' which loudspeaker it goes to investigate).

Ehret's solution to this puzzle is extremely neat. In a second experiment, virgin females without experience of pups were placed in the same apparatus. For a reward of drinking water, they were trained to discriminate between 50- and 20-kHz tone bursts and to approach the source of the former tone in order to receive their reward. After conditioning to criterion with binaural presentation of the two simultaneous tone bursts, these animals were then likewise tested with either the left or the right ear plugged. The conditioned response to the 50-kHz tone persisted irrespective of which ear was attenuated.

When listening with either the left or the right ear, the mice can thus discriminate between the tones, locate the source of the preferred tone and respond appropriately by approaching that source. The cerebral hemispheres of the female house mouse seem equally adept in sound perception *per se*. It is only in the first experiment, where one of the sounds has an intrinsic biological significance (and that significance is known to the mouse) that the left hemisphere so dramatically outperforms the right. Furthermore, the 50-kHz distress call plays a crucial communicative role in the interaction of the mouse mother with her young. Ehret accordingly speculates that an underlying lateral specialization for the auditory perception of communicative signals (between conspecifics) might be "a possible basis of the left-hemisphere advantage for speech sound recognition in man".

The evolutionary story must, however, be a good deal more complicated than this account suggests. In the human brain, it seems that the right hemisphere is specialized for the perception of affective aspects of communication, in both the visual and auditory modalities. Thus the recognition and interpretation of facial gesture[4], and of the emotive significance of prosodic information in speech[5], are more severely impaired by right than by left hemisphere damage. The perception of speech in terms of phonetic and phonemic categories is admittedly a strictly left-hemisphere function[6], and one which is furthermore present in very young infants (as indexed by a right-ear advantage for the perception of speech sounds[7]). Yet there is good evidence that the right hemisphere can process speech in a non-phonological mode and may have semantic access to the meaning of auditorily presented words[8]. The linguistic specialization of the left hemisphere in man clearly extends well beyond the auditory domain; the mechanisms responsible for the expression and comprehension of gestural languages (such as American Sign Language) are as firmly committed to left-sided cortical areas as is any aspect of auditory communication[9].

Nonetheless, Ehret has provided an important demonstration of a left-hemisphere advantage in an animal whose rank in the evolutionary scale might have led one to expect a functionally symmetrical brain. But why nature should choose an asymmetrical location for critical biological functions remains as mysterious as ever. The customary account which is given for lateralized control of human speech (or complex song production in canaries and chaffinches) — that it would be very difficult to ensure the requisite synchrony of timing in the operation of centres that were duplicated in two hemispheres — seems not to apply to the perception of 50-kHz tone bursts. ☐

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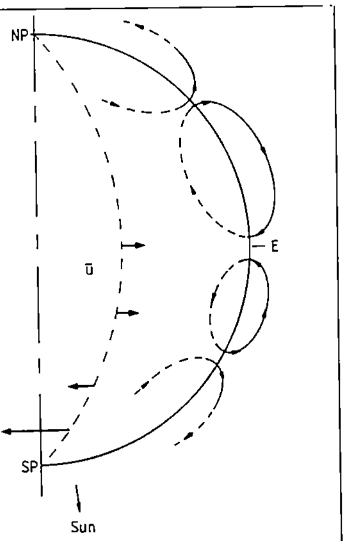
Planetary atmospheres

Clearer circulation on Uranus

Peter L. Read

THE SPACECRAFT encounters by Voyagers 1 and 2 of Jupiter and Saturn in 1979 and 1981 yielded much new information on the upper-level circulations of their atmospheres. But the data were insufficient to resolve an earlier dilemma concerning the dominant energy source for the observed atmospheric motions. Both Jupiter and Saturn possess considerable interior sources of energy associated with continued gravitational contraction and differentiation which are capable in principle of driving an extremely deep-seated circulation. In addition, solar radiation affects a shallow surface layer. To unravel the dynamical implications of these two energy sources, some kind of controlled experiment was clearly desirable. One such opportunity was provided in January 1986 in the encounter by Voyager 2 of Uranus, a planet known to possess at most only a weak interior energy source, and whose solar heating would be strongly affected by the unique obliquity of its rotation vector to that of its orbit (98°). The initial results from this encounter (published as a special issue of *Science* **233**, 39–109; 1986) provide many new clues concerning the form of the circulation of Uranus, but also indicate several puzzling inconsistencies. From more detailed analyses of these measurements, presented at a recent meeting*, it is clear that several of these questions have been resolved, and a better picture of the atmospheric circulation of Uranus is beginning to emerge.

Preliminary infrared data had suggested scarcely any horizontal thermal contrast with latitude, despite the north pole having been in darkness for more than 40 Earth years. This had been anticipated[1] to result from the immensely long thermal inertia of the atmosphere, and was confirmed in some detailed radiative–convective calculations including seasonal effects (B. Bézard, Observatoire de Paris, Meudon). The solar-driven component of its circulation must therefore respond to the annual mean distribution of solar radiation, which is a maximum near both poles on Uranus, because of its obliquity. The simplest response to such a non-uniform distribution of heating is an axisymmetric circulation, with ascent in the region of strongest heating and descent where it is weakest, as occurs in the well-known Hadley circulation in the tropics of the Earth. For Uranus, however, this circulation should be in the opposite sense to that of the Earth (see figure), with the atmosphere descending at the equator. Evidence in support of this form of circulation came from Voyager IRIS observations (B.J. Conrath, NASA Goddard Space Flight Center) showing small but vertically coherent temperature differences between one latitude circle and the next, which could be interpreted in terms of heating (or cooling) caused by adiabatic compression (or expansion) associated with downwards (upwards) vertical motion.



Schematic cross-section of the solar-driven axisymmetric circulation on Uranus at the time of the Voyager 2 spacecraft encounter (NP, north pole; SP, south pole; E, equator; ū, cloud-top zonal flow with respect to the rotating planet). The main Hadley circulation comprises the two cells adjacent to the equator. Uranus rotates clockwise as seen from above the north pole.

The transport of angular momentum by such a circulation would be expected[2,3] to have a strong bearing on the distribution of zonal (east–west) motion in the atmosphere of Uranus. Angular momentum would be transported polewards and vertically downwards by a reversed Hadley circulation, spinning up mid-latitudes relative to the tropics. The downwards vertical motion over the equator would deplete the upper (observable) atmosphere of angular momentum, resulting in a net tropical sub-rotation with respect to the rest of the atmosphere.

No further direct measurements of zonal motion were presented at the meeting beyond the lamentably few cloud-tracked wind measurements already available. Some remarkable indirect measurements of zonal motion near the equator have been made, however (G.F. Lindal, NASA Jet Propulsion Laboratory), using data from the Voyager radio occultation experiment at latitudes between 2 and 7 °S. By measuring the latitudinal curvature of constant pressure surfaces and comparing it with the local geoid, Lindal and colleagues could infer that the equatorial region of Uranus rotates more slowly than the magnetosphere (generally assumed to be tied to the electrically conducting deep interior), with a rotation period of about 18 hours (compared with the magnetic period of 17.24 hours). Thus, Uranus seems to be unique in the Solar System in possessing a retrograde equatorial jet near the top of its atmosphere. The sense of the geostrophic 'thermal-wind' shears[5,6] in the tropics of Uranus, which are inferred from the horizontal temperature gradients, are therefore brought into line with similar measurements on Jupiter and Saturn, so that the pattern of zonal winds on all three major planets of the Solar System evidently decays with height above the visible clouds.

It seems inescapable that the solar-driven component is the dominant energy source driving the upper-level circulation of the atmosphere of Uranus. An assessment of the significance of this result for the other major planets, however, must await further refinements to measurements of the interior heat source of Uranus. It is reassuring to find that the observations of winds and temperature on Uranus (as on Jupiter and Saturn) can be interpreted within a coherent framework based on zonally symmetrical circulations, but several intriguing questions still remain.

The physical reason why all wind systems on the major planets decay with height above the visible cloud decks is still far from clear, although it probably entails the effects of momentum transfer by eddies. There is little direct evidence of such eddies in the atmosphere of Uranus, yet theoretical analyses[4,5] of idealized prograde jets, such as found on the planet at mid-latitudes, suggest a strong propensity for such instabilities, much as in the troposphere of Earth. The presence of prograde flow with respect to the magnetosphere (and hence, by assumption, to the deep interior) at latitudes polewards of the equatorial-reversed Hadley circulation in each hemisphere probably indicates the presence of a further circulation cell at higher latitudes (as shown in the figure), although the mechanism for such an indirect circulation, with descending motion implied near the sub-solar point, is not yet apparent.

The circulation pattern inferred from the infrared data is evidently asymmetrical between the two hemispheres, in the sense that the entire pattern seems to be displaced along the rotation axis towards the (dark) north pole. This apparent displacement is in the same sense as that of the dipole component of the magnetic field of Uranus (F.M. Flasar, NASA Goddard Space Flight Center), the centre of which is located well away from the centre of the planet. Such a coincidence hints at the possibility, speculated on by D.J. Stevenson (Caltech), of magnetohydrodynamic coupling between surface motions and the electrically conducting interior (as also suggested[6] for Jupiter and Saturn), although details of such an interaction remain to be explored. ☐

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* Division of Planetary Sciences of the American Astronomical Society meeting, Paris, 4–7 November 1986.

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Nomadic agriculture

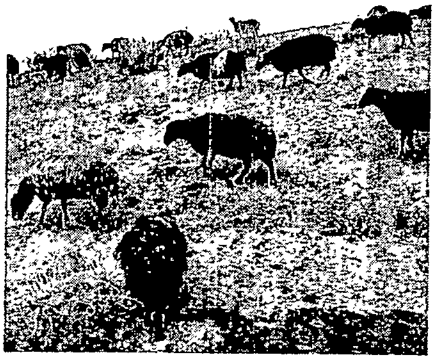
Mobile resources for survival

Peter D. Moore

NOMADIC pastoralism in the semi-arid regions of the world is a hazardous occupation because of the unpredictability of water supply and therefore the unreliability of forage production[1]. The vegetation of such areas is often patchy[2] but contains a range of species that vary in their drought tolerance and in their palatability to herbivores[3]. Under such circumstances it could be advantageous for pastoralists to maintain mixed flocks of grazing animals so that resource use can be kept at a maximum even when those resources are depleted through drought. Coppock, Ellis and Swift[4,5] have recently examined this proposal by studying the food intake of the various components of mixed flocks of animals in Kenya and conclude that the use of several species, combined with the mobility inherent in the nomadic way of life, does indeed enhance the efficiency of grazing systems and increase the likelihood of survival in times of drought.

Vegetation in semi-arid lands varies both in space and time. Plant biomass and productivity generally increase with water supply and a range of plant forms may contribute to this, including shrubs (or even trees like *Acacia*), perennial grasses, bulbous perennials, and annual grasses and herbs. Some of these, particularly the annuals, may be available for grazing only for a short period each year during the wet season so that variability occurs in time as well as in space[6]. This fluctuating plant biomass forms the resource base on which pastoralism is founded.

The dietary preferences of the animals used by pastoralists can be studied by analysing the gut contents of dead animals[7], by surgically examining the living (fistulation), by fecal analysis[8] or by detailed observation of grazing behaviour. Analysis of the gut contents is wasteful and fistulation involves many practical problems in a nomadic herd. There are also several problems with fecal analysis, including differential digestion and the problems of identification when dealing with a diverse array of plant cuticles, hairs and so on[9,10]. The final option, observation, requires the logging of the number of bites a particular animal takes from each plant species in a given time, together with the determination of bite size for that animal. Measuring bite frequency and at the same time identifying the prey plant can be quite difficult, especially with some fast eaters; many grazers, like sheep, cattle, deer and kangaroo have a rate of about 50 bites per minute, but some speedier ungulates, such as reindeer, can exceed 200 (ref. 11). It takes a sharp eye to identify lichens at that speed.


Good mixers: sheep flock in north-east Iran.

Using this method of diet analysis it is possible to establish how domestic grazers exert their preferences and thus partition the resources of the environment between them[12]. Nyerges[13], for example, studied the grazing behaviour of sheep and goats in the semi-arid scrub of eastern Iran and found that although the demands of the two animals are indistinguishable as far as herbaceous materials are concerned, there is little overlap in their browsing activites on the shrubs. Goats exploit the *Pteropyrum* and *Amygdalus* scrub in the dried river beds whereas sheep are prepared to consume saline-tolerant succulents like *Salsola*. The one overlap is the small shrub *Artemisia herba-alba*, which is eaten by both sheep and goats (17 per cent of goat diet and 23 per cent of sheep diet).

The studies of Coppock *et al.*[4,5] were conducted in the arid northwestern region of the Kenyan Rift Valley among savanna vegetation, where the average net annual above-ground productivity is 167 g m^{-2}, which compares poorly with the 1,071 g m^{-2} from the grasslands of the Nairobi National Park[14]. The nomadic people of Ngisonyoka Turkana use several different domesticated grazers to exploit this poor productivity. Cattle are specialists in grass consumption (96 per cent of diet), whereas at the other end of the spectrum camels mainly browse on shrubs and trees (95 per cent). Between these extremes, sheep, goats and donkeys enjoy a more mixed diet.

The different types of forage vary in their nutritional value throughout the year, grasses becoming particularly poor in value during the dry season. This means that cattle fail to obtain green fodder within a month of the end of the short wet season. Camels, on the other hand, maintain some green input in their diet throughout the year by feeding on shrubs and trees with deeper roots and hence a more reliable water supply[6]. The more versatile habits of sheep and goats also permit some variety in diet as they can switch from herbs to shrubs when the former become more scarce or less nutritious. Overall, cattle consume more fibre whereas camels select for materials higher in protein and dietary cell solubles, but this leaves them with a relatively low energy intake.

The specialization of diet, both spatial and temporal, observed in these animals is reminiscent of that seen in the natural grazers of the area[15,16] and is clearly well adapted to maximize use of resources. Coppock *et al.* point out that there are considerable advantages in maintaining this diversity of grazing animals used in the traditional pastoral systems rather than encouraging a concentration on a single species. Equally, the mobility which is an inherent feature of the nomadic system should be allowed to continue in order to provide opportunities for spatial as well as temporal variations in diet. ☐

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Number theory

Geometry finds factors faster

Ian Stewart

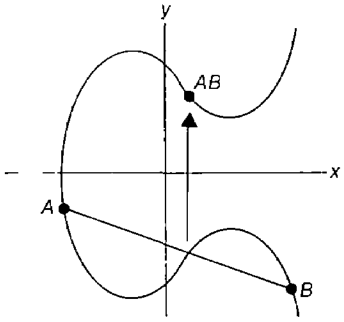
Every whole number can, in theory, be resolved into prime factors. In practice the process may take longer than the lifetime of the Universe. The obvious method, trial division by all primes up to the square root, is hopelessly inefficient. Its execution time grows so rapidly that improvements of a factor of, say, 100 in computer speeds will add only a few digits to the size of numbers that can be dealt with. To factorize an arbitrary 250-digit number — that is, one without special features that make it easy to factorize — is currently out of the question.

On the other hand, nobody has ever proved that prime factorization really must be as hard as it seems to be. Efficient and effective methods have not been ruled out. A new method, devised by Hendrik Lenstra of the University of Amsterdam and based on deep ideas from algebraic geometry and number theory, points to a new direction for attack (*Factoring Integers with Elliptic Curves,* Mathematical Sciences Research Institute preprint, Berkeley, 1986). The problem is of practical importance because of its relation to public-key cryptosystems — methods, hoped to be 'unbreakable', for putting messages into code. If a fast-factor algorithm exists, some of these methods will be breakable. The problem also has intrinsic mathematical interest as one of the most basic questions in number theory.

The objective is an algorithm: a prescribed procedure, involving no guesswork, and guaranteed either to produce a factor or to show that the number is prime. The efficiency of an algorithm is measured by the way its worst-case 'running time' grows with the number n of digits of the number being factorized. The running time is usually defined as the number of arithmetical operations (+,× and so on) involved, and only a relatively crude estimate is sought. A 'good' algorithm runs in polynomial time, growing no faster than some fixed power of n; a bad one runs in exponential time, growing as the nth power of some constant.

For an n-digit number of size roughly 10^n, trial division requires about $10^{n/2}$ trials. Even at one arithmetic operation per trial (which is far too low an estimate) this has exponential growth, hence is inefficient. In contrast, the euclidean algorithm for finding the least common multiple (l.c.m.) of two n-digit numbers takes about $5n$ steps. So this runs in polynomial time and is highly efficient.

To illustrate the alternatives to trial division, I will describe Pollard's $p-1$ method (see Riesel, H. *Prime Numbers and Computer Methods for Factorization,* Birkhäuser, Boston, 1985). This method starts with a number N and looks for prime factors p such that $p-1$ has only 'small' prime divisors. It is based on a number-theoretic fact: if $p-1$ divides a number Q and p does not divide Q then p divides a^Q-1 for any a not divisible by p. Then the l.c.m. of N and a^Q-1 is divisible by p, and this can be found rapidly by the euclidean algorithm. In practical implementations a standard series of Qs is defined and stored in the computer: for example, Q = l.c.m.$(1,2,\ldots,r)$ for $r = 1,2,3,\ldots$ up to some predetermined limit. As an example, the method has been used to find the factor $p=2670091735108484737$ of $3^{136}+1$. Although p is large, $p-1$ has the factorization $2^7.3^2.7^2.17^2.19.569.631.23993$, and the primes involved there are all small.



The group law on an elliptic curve. Given two points A and B, form the line joining them. This cuts the curve in a third point. Reflect that point in the horizontal (y) axis to get the point AB, which is the 'product' of the original points A and B.

The key to Lenstra's method is to view Pollard's algorithm in more abstract terms. Suppose that p is a prime. If any two of the set of numbers $1,2,\ldots,p-1$ are multiplied together modulo p (that is, the remainder is taken on dividing the product by p) then another number in that set is obtained. The set is said to form a group under multiplication modulo p. Pollard's method can be seen as exploiting the structure of this group. Lenstra's method is obtained from Pollard's by replacing the group by a more subtle one: the group of points on an elliptic curve.

Elliptic curves have been studied by number-theorists for about a century; not for applications to prime factorization, but because of their intrinsic mathematical beauty and interest. They are curves defined by an equation of the form $y^2 = x^3+ax+b$, for constants a and b. Associated with any such curve is a geometric multiplication law. Given any two points A and B on the curve, draw the line between them (see figure). Because the curve has an equation of degree 3 this line meets the curve at a third point. Multiply the y-coordinate of this third point by -1: because of the term y^2 in the equation, the result is still a point on the curve. Call it the 'product' AB of the first two points. The surprise is that this product obeys sensible algebraic laws: the points on the curve form a group.

In Lenstra's algorithm the coordinates x and y are taken to be integers modulo N, where N is the number to be factorized. If a single elliptic curve is used the method is much like Pollard's. However, the number $p-1$ that occurs in Pollard's method is replaced by the number $p-t$ where t depends on the choice of elliptic curve. Different curves give different ts, so there is a whole range of possible numbers $p-t$. As long as just one of these has small divisors, the method will find a factor.

The algorithm therefore starts by choosing some elliptic curve and seeking a factor by a generalized version of Pollard's method. If this fails, it does something that is not available in the original Pollard method: it tries another elliptic curve. Provided there is a reasonably dense set of numbers near p that are a product of small primes, the method will find a factor rapidly. The precise running time is rather complicated, and its proof depends on a plausible but unproved conjecture on this density. Other known methods have a similar conjectured running time, but they lack one important feature of Lenstra's method: the running time depends on the size of the prime factors of N.

Until very recently nobody had expected there to be any connection between elliptic curves and prime factorization. Only with hindsight is the connection clear. Although Lenstra's method may seem complicated, it is a natural generalization of Pollard's method. Perhaps even more efficient primality tests lurk unsuspected among the discoveries of number theorists. If so, some deep and abstract mathematics will take on a new, practical guise. Computer scientists working on algorithms for factorization would be well advised to brush up on their number theory. ☐

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Erratum

In the article 'Melting and the surface' by Robert Cahn (*Nature* **323**, 668; 1986) a sentence in the sixth paragraph was omitted. The second half of the paragraph should have read: "The melting temperature of argon at atmospheric pressure is 84 K, but allowing for the pressure in the bubbles (estimated from the measured anomalously small lattice parameter of the argon) it should be about 250 K. In fact, the diffraction pattern of solid argon is detectable up to 730 K, that is, a metastable superheating of 480 K!"

Evolutionary adaptations

Aristotle's theory of mammalian teat number is confirmed

Jared M. Diamond

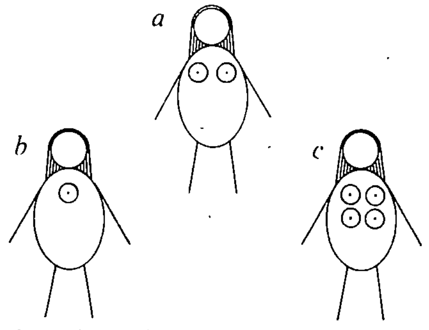
Animals which have small litters, both those that have solid hoofs and those that carry horns, have their mammae by the thighs; and there are two of them. Animals that have large litters or are polydactylous . . . have numerous mammae placed at the sides upon the abdomen — for example, swine and dogs. (Aristotle, *The Parts of Animals* Lesbos, about 350 BC).

Le nombre des mamelles et leur situation sont extrêmement variables . . . Il est d'ailleurs ordinairement en rapport avec le nombre des petits que les femelles peuvent mettre bas. (G. Cuvier, *Leçons d'Anatomie Comparée* vol. 5, p.155; Baudoin, Paris, 1805).

MAMMARY glands are so central to ecology and life history in the order Mammalia that they give the order its name. We think anthropocentrically of the normal teat number as two but, as Aristotle noted, it varies greatly among species: some primates have six, and rodents have up to fourteen. What accounts for this interspecific variation? Why, for instance, does the human female have design *a* rather than *b* or *c* (see figure)?

Aristotle suggested that teat number is related to litter size, a view followed by Cuvier but varously espoused or disputed by twentieth century scientists. A recent analysis by A.N. Gilbert (*Proc. natn. Acad. Sci. U.S.A.* **83**, 4828–4830; 1986), resting on a massive database, now supports Aristotle. Gilbert assembled values, for 266 rodent species, of teat number T (ranging from 2 to 14), mean litter size $\bar{L}$ (ranging from 1 to 8) and maximum litter size L_{max}. There is a significant positive correlation between $\bar{L}$ and T: $\bar{L} = 0.39 \pm 0.46T$, $P < 0.001$, $r = 0.72$. Averaged over all species, the ratio $\bar{L}/T$ is 0.53 ± 0.01, and L_{max}/T is 0.92 ± 0.02. Thus, there are two teats per pup for an average litter, one for a maximal litter.

This observed correlation begs the question as to cause and effect: does litter size determine teat number or vice versa? At a proximate level, teat number does affect surviving litter size, as milk production is critical to pup survival and there is a sharp increase in pup mortality when litter size exceeds teat number. The value $L_{max}/T = 0.92$ means that natural selection has produced mammals with a safety margin of teats, enough to provide one per pup in the occasional large litter.



Theoretical designs for human mammary gland arrangement. *a*, The accepted number; *b*, no excess capacity for twins; *c*, too extravagant.

At an ultimate level, there have been many theoretical discussions of the evolution of litter size, which is generally seen as constrained by factors such as latitude, length of breeding season, body size and metabolic rate. By ignoring teat number, such analyses tacitly assume that it easily evolves in response to the driving variable of litter size. However, Gilbert suggests that this may not be true. For instance, within a few generations it is feasible to select animals for increased litter size but not for increased teat number, which is a rather constant species characteristic.

If we now return to our question concerning human females, they are seen to fit Gilbert's rodent-based theories perfectly. For $T = 2$, the relations $\bar{L} = 0.53T$ and $L_{max} = 0.92T$ predict that our maximum litter size should be 1.84 (after rounding to an integer, twins) and our mean litter size 1.06 (usually singletons). In fact, singletons are our norm, twins a not uncommon exception (5 per cent of all births in some populations) and triplets vanishingly rare. Thus, our selected design *a* in the figure is to accommodate the occasional twin birth, whereas the other designs are rejected because they lack a safety margin (*b*) or are extravagant (*c*). ☐

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Edward A. Doisy (1893–1986)

EDWARD A. DOISY died on 23 October 1986, one month before his 93rd birthday. His numerous scientific achievements centred around his masterful selection of methods for the isolation of trace natural products of biological significance. Needless to say, the methods available to Ed Doisy in the early part of this century were crude compared to those available today. His major contributions were made while residing in the city of St Louis, where he worked first at Washington University (1919–1923) and then at St Louis University (1923–1965).

Edward Doisy was born in Hume, Illinois, on 13 November, 1893. He studied chemistry at the University of Illinois and then went to Harvard to work under the guidance of Otto Folin, receiving his doctoral degree in 1920.

He was appointed an instructor in biochemistry at Washington University School of Medicine in 1919 and became associate professor in 1923, when he moved across town to become the first professor and chairman of a new department of biochemistry at St Louis University, a position he held until his retirement in 1965.

At Washington University he began his work on the isolation of natural products by the purification of insulin by isoelectric precipitation. He then developed an interest in ovarian function and the nature of the ovarian hormone through collaborative studies with the anatomist Edgar Allen. By judicious application of bioassays for oestrus in rats and his skill in the purification of trace compounds, he was the first to obtain oestrone, one of the female sex hormones, in crystalline form from urine in 1929. A few years later, he isolated from several tons of sow ovaries the more potent ovarian hormone oestradiol. These discoveries opened up the field of fertility to biochemical study, the consequences of which are still being exploited.

In 1936, following the discovery of vitamin K as a new fat-soluble compound that promoted coagulation of blood in chicks and rats by Henrik Dam, Doisy undertook the isolation of this lipid vitamin from various sources, including alfalfa and fermented fish meal. In 1939, he succeeded in crystallizing vitamin K from alfalfa meal and vitamin K_2 (now called menaquinone-7) from fermented fish meal. He subsequently synthesized homologues in both the vitamin K_1 and K_2 series. Doisy received the Nobel Prize in physiology and medicine, jointly with Dam, the discoverer of vitamin K_1, in 1943 for his work on the isolation and synthesis of vitamins K_1 and K_2. No doubt his contributions to the oestrogen field played some role in the award.

During World War II, he worked for the Office of Scientific Research and Development and isolated several new antibiotics. After the end of the war, Doisy began to study bile acids and succeeded in isolating novel bile acids from the rat.

His many students and associates remember Edward Doisy as a single-minded and astute judge of research problems that could be solved with the techniques available, and an excellent mentor who both recognized scientific skills in young people and helped to develop them to the utmost. Robert E. Olson

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Bone nitrogen isotope composition and climate

SIR—Heaton *et al.*[1] report that the ^{15}N/^{14}N ratios of bone collagen from prehistoric humans with little or no access to marine protein show a negative correlation with present rainfall levels in the areas of South Africa they inhabited. Such a correlation cannot, however, be accepted uncritically, because the influences of dietary differences among such populations have not been considered. For example, ^{15}N/^{14}N ratios of bone collagen of early historic Griqua pastoralists from the arid interior of Orange Free State are higher than those of late prehistoric Iron Age farmers of the Northern Transvaal[2]. This is expected given the well-known trophic level effect on nitrogen isotope ratios of bone collagen[3]. The data for these two populations, if plotted together against rainfall, would produce a regression similar to that obtained by Heaton *et al.*, but would not reflect only climatic influences. Thus the authors' interpretation of the nitrogen isotope ratios for human bone collagen based strictly on climate seems to be premature.

By contrast, their data for modern herbivores demonstrate a correlation between rainfall levels and the nitrogen isotope ratios of bone collagen that cannot be ascribed to dietary differences because no relationship between plant ^{15}N/^{14}N ratios and rainfall was observed[1]. The authors suggest that the higher ratios of "mammals in arid areas are therefore more likely to be linked with the nitrogen metabolism in the body of the animal itself". In the absence of more specific explanations we feel compelled to summarize our own model and correct a potential flaw in its original presentation[4].

The enrichment of ^{15}N in the bone collagen of East African water-conserving herbivorous mammals relative to water-dependent ones that we reported suggested a relationship between physiological mechanisms of water conservation and nitrogen isotopic mass balance[4], as follows. Urea, the major form in which nitrogen is excreted by mammals, is known to perform an essential function in urinary water conservation[5]. Herbivorous mammals on diets high in protein have the capacity to excrete a highly concentrated urine under conditions of heat and water stress. This is accompanied in most species by an absolute and sometimes spectacular increase in urea output, as in the dikdik[6] and two cattle breeds[7], though not in the camel[8] or in cattle on low-protein diets[7]. Urine in *Bos taurus* has been shown to be depleted in ^{15}N relative to the diet, with the depletion being greater during the day than at night[9] (when water stress is presumably lower). If this difference occurs in all mammal species, animals under more continuous water stress would respond by excreting a more concentrated urine with a quantitative increase in the excretion of ^{15}N-depleted urea, resulting in higher ^{15}N/^{14}N ratios in the unexcreted nitrogen. In order to satisfy mass balance constraints, the tissues synthesized from this remaining nitrogen pool in water-conserving, urea-excreting mammals should have higher ^{15}N/^{14}N ratios than those of mammals that do not conserve water and/or intensively recycle urea. Such a mechanism would explain the nearly one-to-one correlation in rank order we have found between maximal urinary osmolality[7] and mean bone collagen ^{15}N/^{14}N ratios for zebu cattle, donkey, impala, goat, sheep and dikdik[4], and most of the data for herbivores presented by Heaton *et al*[1].

This model is only a hypothesis but it can be tested by controlled water-stress experiments and analysis of nitrogen isotopic mass balance in animals living in different climates and microhabitats. Until the contributions of ecological and physiological processes to variation in animal tissue nitrogen isotope ratios are more clearly understood, palaeodietary analysis based on ^{15}N/^{14}N ratios of bone collagen must be regarded as uncertain in all but the simplest of ecological contexts.

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Signal-transduction

SIR—Wakelam *et al*[1], as discussed in News and Views by Petersen and Bear[2], have demonstrated that although concentrations of glucagon above 10^{-8} stimulate the intracellular concentration of cAMP but not of inositol phosphates in hepatocytes, low concentrations (10^{-9}M) increase inositol phosphates but not cAMP. We would like to draw attention to the corresponding effects of ACTH on cells from the rat whole adrenal cortex; concentrations of ACTH above 10^{-10} M stimulate cAMP levels but neither ^{32}P incorporation into phosphatidic acid and phosphatidylinositol nor the production of inositol phosphates, whereas $\sim 10^{-12}$ M ACTH produces the opposite results[3,4]. Moreover, we have obtained similar results using rat zona fasciculata–reticularis (ZF–ZR) adrenocortical cells[5,6] and have shown that high concentrations of ACTH inhibit the increase in production of inositol phosphates by angiotensin II in ZF–ZR cells[7].

Wakelam *et al.*[1] also report that TH-glucagon, an agonist for all the biological effects of glucagon, is effective in stimulating the production of inositol phosphates but not the intracellular concentrations of cAMP. There are also ACTH analogues that are effective in stimulating steroidogenesis in ZF–ZR cells but are only weakly active in activating adenyl cyclase[8]. Their activity in stimulating phospholipase C would now be of interest.

The steroid output of ZF–ZR cells is stimulated mainly by ACTH-like peptides but zona glomerulosa (ZG) cells of the adrenal cortex, which produce aldosterone, are stimulated by many different factors. These factors employ either the adenyl cyclase or phospholipase C system[6]. There are as yet no reports of ZG stimulators acting through both systems in the concentration-dependent manner of ACTH in ZF–ZR cells. This would be an appropriate area for further study.

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Achieved spacetime infinity

SIR—In his review[1] of my book with John Barrow, *The Anthropic Cosmological Principle*, Press accuses us of indulging in "a distressing amount of mathematical flim-flan . . . For example, . . . Barrow and Tipler [claim] 'A Penrose diagram allows us to define rigorously "an achieved infinity", a concept whose logical consistency philosphers have been doubtful about for thousands of years'. This is a silly assertion, but it is put forth with the utmost gravity, in such a way that many readers will be taken in. And it is only one of many such cases." I cannot comment on the "many such cases" which are not given in the review, but the "silly assertion" is, perhaps, a case of misunderstanding. Philosophers have debated whether the notion of 'an achieved infinity,' is a meaningful concept since Aristotle questioned the notion in his *Phyics*; see Sorabji[2] for a discussion and a history of the debate up to the Middle Ages. The debate is still current; Popper and Wittgenstein[3] had a sharp exchange on the question in 1946.

As one will see in the context of the book, we meant by an 'achieved infinity' a spacetime singularity. The Penrose diagram, or more generally the c-boundary construction, allows us to define a spacetime singularity in a mathematically precise way[4] by attaching a boundary to spacetime. The c-boundary construction is very elegant and natural for globally hyperbolic spacetimes, the only class of spacetimes we considered. In the c-boundary construction, a regular spacetime point is identified with the past light cone $I^-(\gamma)$ of a future-directed time-like curve γ that terminates in that point, and the future c-boundary points are identified with the past light cones $I^-(\gamma)$ of future-directed time-like curves that have no future endpoints in the spacetime. (Past c-boundary points are defined analogously using future light cones.) If a time-like curve defining the c-boundary point is incomplete (that is, of finite length) the point is said[5] to be a 'singular c-boundary point'. These future singular c-boundary points are the future singularities.

It is generally accepted among relativists that if in fact the Universe is closed and terminates in a final singularity in finite proper time, then the Universe will actually reach this singularity. Strictly speaking, this is not true, since the c-boundary points are not in the spacetime, but there seems to be a general consensus to stretch the meaning of the word 'reach' in this case. Thus the final singularity really exists if the model of gravitational collapse is accurate. From the point of view of the c-boundary construction, the ontological status of the regular spacetime points and the future c-boundary points is the same: they are both the past light cones of time-like curves. Thus if one set of points really exist, then so does the other, and we agree that spacetime really exists. Furthermore, if the initial c-boundary of the Universe is an initial singularity, we can receive information from this singularity just as we can receive information from any other regular spacetime point in our past light cone. Thus it seems reasonable to say it really exists, if such is the initial c-boundary.

The singular c-boundary points are reasonably thought of as infinite because they are (in general) points at which physical quantities are actually infinite. Thus spacetime singularities — if they are indeed on the c-boundary of the actual Universe — are an achieved infinity and are precisely defined by the Penrose construction. I would have thought that this assertion is the overwhelming consensus of the relativity community.

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Estimating guild sizes

SIR—John Lawton[1] has drawn attention in News and Views to the important simulation model of Shorrocks and Rosewell[2] which assumes that aggregation of individual *Drosophila* species is reflected through the parameter k of the negative binomial distribution. The model is based on successive sums of such distributions for two species. The resulting summed distribution is assumed to be negative binomial also, with parameter k_s, equal to the sum k_1+k_2 of those of the component species.

Shorrocks and Rosewell correctly note that this is precisely true only if $\mu_1 k_2 = \mu_2 k_1$, but claim that with equal mean values of 10 or more and values of $k<2$, it is a close approximation. This claim is technically wrong. Assuming the summed distribution is approximately negative binomial, and that the component species have equal mean values, the method of moments estimator of k_s may easily be found to be

$$\hat{k}_s = \frac{4k_1k_2}{k_1+k_2}$$

The approximation does not depend upon the mean value, and the mean value does not need to be 10 or more.

More importantly, however, unless k_1+k_2 is close to $\hat{k}_s$ the approximation will be poor. To see the scale of error involved take two examples: first, $k_1 = 7/4$, $k_2 = 1/4$ gives a claimed k_s of 2 compared with estimated $\hat{k}_s$ of 7/8; second, $k_1 = 2$, $k_2 = 1/2$ gives a claimed k_s of 5/2 compared with estimated $\hat{k}_s$ of 8/5. Both examples show the approximation is not at all close. The effect that this and the assumption of equal mean values will have on the model requires thorough investigation, although it appears the correction would generate larger estimates of guild size than those estimated by Shorrocks and Rosewell which, as the authors themselves noted, were not quite as large as values from their field studies.

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Aluminium leaching from cooking utensils

SIR—Aluminium is known to have neurotoxic effects[1-3]. With the discovery that abnormally high levels of aluminium are present in senile plaques in Alzhimer's dementia[3-5], the cumulative effects of aluminium poisoning and the question of how this metal enters the body become problems that need immediate attention. Recently, Coriat and Gillard have drawn attention to the high aluminium content of tea leaves and their infusions; aluminium compounds can also be found in water and can be released from utensils during cooking[3-7]. We have found that the leaching of aluminium from utensils is dramatically enhanced in the presence of trace quantities of fluoride ion.

In an experiment conducted to estimate the rate of leaching, we have found that the presence of only 1 p.p.m. of fluoride (the permitted level of fluoridation[8]) in water adjusted with citric acid or sodium bicarbonate to *p*H ~ 3 (a *p*H often realized in cooking conditions) and boiled in an aluminium vessel, liberates nearly 200 p.p.m. of aluminium in 10 min, compared with less than 0.2 p.p.m. in the absence of fluoride. Prolonged boiling produces a concentration of ~ 600 p.p.m., which is reached more quickly the larger the surface-to-volume ratio of the water. The rate of dissolution is *p*H-dependent with a minimum at neutral *p*H. Crushed tomatoes (50g in 250 ml) cooked in the same vessel with 1 p.p.m. of fluoride produced a concentration of ~ 150 p.p.m. of aluminium in 10 minutes. Water consumed in some localities contains 10 p.p.m. or more of fluoride. And cooking or prolonged storage in aluminium ware of foods that contain large amounts of fluoride[9] (~ 500 p.p.m. in tea, 100 – 700 p.p.m. in fish) could easily release more than 100 p.p.m. of aluminium.

The normal resistance of aluminium to corrosion in mildly acidic or alkaline solutions depends on the formation of an inert oxide film. The corrosion in the presence of fluoride perhaps results from a permeability of the oxide film to fluoride ions, which disrupt the protective film.

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A merchant of light

R. V. Jones

The Griffin: The Greatest Untold Espionage Story of World War II.
By Arnold Kramish. *Houghton Mifflin: 1986. Pp. 294. $17.95.*

"THE GRIFFIN" of this book's title was Paul Rosbaud, who, as science adviser to the great German publishing house of Springer in the years before 1939, was acquainted with many scientists throughout Western Europe. He travelled widely and frequently, both in search of authors and in promoting the firm's publications.

Rosbaud, who had been born in 1896 and brought up as a Catholic, served with distinction in the Austrian Army in the First World War, after which he became a physics student in Berlin and took a doctorate at the Technische Hochschule. He then moved into publishing, joining Springer in the early 1930s. Alarmed by the rise of the Nazis, concerned by their treatment of his Jewish friends and holding a deep faith in the international character both of science and of the humanities, he used his many contacts to do all that he could to ease the problems of those whose welfare — and indeed lives — were increasingly under threat. And not only those threatened by the Nazis, for in 1935 he also tried to persuade the Russians to let Kapitza return to Cambridge after they had trapped him on a visit to Leningrad.

Paul Rosbaud — "a natural courier of messages between scientific friends across national frontiers;...".

With so many contacts in the world of pre-war science and with frequent opportunities for travel, Rosbaud was a natural courier of messages between scientific friends across national frontiers; and, from 1939 onwards, some of these messages could provide a means of intelligence. Opportunely, he had known and liked a member of the British Intelligence Service, Frank Foley, for some years. Because he remained in Germany, he could maintain a fringeing contact with German research laboratories, and from time to time he succeeded in getting information passed to London.

Rosbaud's life, particularly as an active agent in wartime Germany with ostensible reasons for visits abroad, is thus a promising subject for a biographer. It has now found one in Arnold Kramish, who was at one time a physicist in the American Atomic Energy Commission and at others attached to the Rand Corporation and the London School of Economics. Kramish has assiduously gathered details of Rosbaud's life and has delved into the murky world of Intelligence with considerable success. He has discovered much about Rosbaud that I for one did not know, even though I saw the most crucial of Rosbaud's reports that were successfully transmitted during the war, and though I came to know him fairly well afterwards when he lived in London and he wanted me to be the British editor of the new *Handbuch der Physik* — he had returned to publishing, only to have an unhappy relationship with Robert Maxwell who was, I believe, indebted to him for the name "Pergamon".

The clearest of Rosbaud's wartime contributions for which I can personally vouch was his letting us know about the doings and movements of the German nuclear physicists headed by Werner Heisenberg. Rosbaud was intensely alive to the possible release of nuclear energy, for it was he who had rushed the paper by Hahn and Strassmann on the new elements created by the neutron bombardment of uranium into the columns of *Naturwissenschaften* in early 1939. Rosbaud's wartime reports were particularly valuable because they led us correctly to conclude that work in Germany towards the release of nuclear energy had at no time gone beyond the research stage: his information thus calmed the fears that might otherwise have beset us. Kramish's description of Rosbaud's dangerous work in this connection by itself justifies the biography.

I cannot speak with such confidence about Rosbaud's other contributions to scientific intelligence that Kramish describes. Where he discusses events and characters with which I had direct contact, I sometimes have difficulty in reconciling his account with my own memories. He is handicapped by having to speculate too far from an inevitable sparsity of known facts, particularly on events inside the British Intelligence Service. Some of his speculations seem tendentious, and some of his descriptions are quite erroneous — "Sir Edward Appleton of the S.I.S.", for example, at least if S.I.S. has its normal connotation of the Secret Intelligence Service. Rutherford is surprisingly described as "certainly a theoretician" in contrast with Cockcroft; the V1 flying bomb was not a glider; and, so far as I know, phosphorescent materials are not "an important ingredient of heat-seeking sensors that guide missiles to their targets". Such examples contrast with the punctilious accuracy that Kramish sometimes achieves.

In other places he appears to have aspired to verisimilitude by adding naturalistic detail that he could not possible know — a charge that he himself properly lays in one instance against another author, Anthony Cave Brown. This concerns the mode of delivery of the Oslo Report, the package of information on new German weapons and techniques that was sent to the British Legation in Oslo from an anonymous source early in November 1939. The Report told us, for example, of a new radio ranging system that we later found the German pathfinders using in the Blitz, and of the two principal types of German radar equipment which we later discovered under the code-names Freya and Würzburg; other items included acoustically homing torpedoes, radio-controlled glider bombs, and large rockets with gyroscopic control. Peenemünde was also mentioned. Kramish himself claims to have discovered "beyond doubt" that the Report came from Rosbaud, and that "it seems indisputable" that it was delivered to the Legation by the hand of Odd Hassel, who later won a Nobel Prize.

It can be argued convincingly that Rosbaud had the qualifications and the motive to write the Report. He could perhaps also have had the knowledge. Kramish speculates that Rosbaud had the opportunity to visit Oslo in November 1939; but he has to admit that although Rosbaud was there in September, Rosbaud himself stated after the war that he had no further contact with Hassel until December. So Kramish has to add to the conjectures with which the book is peppered the further one that Rosbaud's memory was at fault.

As for the speculation that Hassel handed the Report into the Legation, Kramish appears to have overlooked the positive statement by the officer who received it, the Naval Attaché, Captain Boyes, that it came in the form of letters "posted in Norway". That statement was available to Kramish, although he does not mention it. Had he done so, he might have proposed that Captain Boyes's memory, like Rosbaud's, was at fault.

Actually there is no need for either speculation, for Rosbaud did not write the Oslo Report: to my positive knowledge he had nothing to do either with its provenance or its transmission. At the same time, despite this substantial error, Kramish has performed a welcome service in ensuring a wider appreciation of those genuine and important contributions that Rosbaud so courageously made. ☐

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Brief encounters

Jeremy K. Burdett

New Directions in Solid State Chemistry. By C.N.R. Rao and J. Gopalakrishnan. *Cambridge University Press: 1986. Pp. 516. £55, $79.50.*

SOLID state chemistry is a tough subject and for many years was rather unfashionable. Synthetic techniques are at the Neanderthal stage compared to those used in organic synthesis. Characterization of the reaction product is invariably difficult, because it is often non-crystalline — in these cases, X-ray crystallography, a major tool for the molecular inorganic and organometallic chemist, is inapplicable, and NMR, a mainstay of molecular organic chemistry, is neither as easy to perform nor as easy to interpret. The materials themselves are frequently non-stoichiometric and often the presence of a tiny amount of impurity can dramatically change the crystal structure. In addition, the description of solid state structures, relating the collection of x, y, z coordinates to some geometrical model in terms of linkages between atoms in close contact, sometimes presents quite a challenge. All of these points emerge from a close reading of Rao and Gopalakrishnan's new book, in which the authors attempt to paint a broad picture of our current knowledge of solid state chemistry. At the moment, the field is experiencing a resurgence of interest from chemists of all persuasions, and the appearance of a work of this type is clearly timely.

New Directions, however, is written as a very long review article. It has the flavour of a Chemical Society *Specialist Periodic Report*, in that a large number of topics are covered in each chapter, many of which are described in a sentence or two and then labelled with a reference. The result is that the book provides only a sketchy overview of the area as a whole, and gives little new insight into some of the tantalizing problems mentioned above.

A further problem for those working on the solid state is how to model the structure of a particular solid in terms of chemical bonds. Chemists have always leaned heavily on structure/bonding relationships, but when it comes to solids, description of the way some of these complex materials hold together is not at all simple. Rao and Gopalakrishnan use the traditional division into solids of different chemical bonding type, and although the shortcomings of the ionic model for coordination number prediction (Pauling's radius ratio rule) are presented, no incisive discussion of this fundamental topic follows. Similarly, although the solid state analogue of molecular orbital theory (tight-binding theory) is mentioned, the pedagogical opportunity presented by this correspondence is not pursued.

Although it is entitled *New Directions*, the book is somewhat backward looking. Much of the material describes recent work using traditional concepts and philosophy. For example, studies of the organic solid state in research on conducting polymers have led to some remarkable collaborations between physicists and chemists, perhaps one of the success stories in this interdisciplinary area. A startling variety of species of semiconducting, superconducting and metallic solids have been produced, the properties of which may often be subtly adjusted by changing pressure or the nature of an apparently innocent atom or grouping in the organic building blocks. Polyacetylene and $(SN)_x$ are now textbook examples of these types of materials, and yet less than 12 lines are used to describe them and the science behind them.

Another area which does not get its due is the electronic structure of solids. Large strides have been made in recent years in understanding the crystal structures of extended arrays, but only passing reference is made to the work of the new breed of physicists, chemists and chemical physicists who are intent on linking structure and properties of materials. For example, it has been possible for at least half-a-dozen years to predict by calculation from a list of possibilities the lowest energy structural alternative (admittedly, for simple systems only).

Altogether, the book would serve quite well as a relatively brief introduction to those new to solid state chemistry. But the authors have missed the opportunity to present some of the novel developments of the past few years and convey the excitement which is now percolating through this area. ☐

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Detail from a painting by Andy Warhol of the California condor; the original is in yellow and red on black. The picture is reproduced from Vanishing Animals, *an account in words (by Kurt Benirschke) and pictures (by Mr Warhol) of the plight of 15 species of endangered animal. Publisher is Springer-Verlag, price is DM 120, $49.50.*

Culture in danger

Terence R. Lee

Risk Acceptability According to the Social Sciences. By Mary Douglas. *Russell Sage Foundation: 1986. Pp. 115. Distributed by Basic Books, New York, pbk $6.95.*

RISK assessment and the concept of acceptable risk are much in vogue of late, and there are few learned scientific societies that have not felt the need to confer on the subject. But this book is not in the mainstream of such considerations and does not mention probabilistic risk assessment, fatal accident frequency rates or LD_{50} tests. Rather, it is about risk *perceptions* as products of culture.

In the past, approaches to risk through the social sciences have been largely dominated by psychometric studies of perceived risk and by classical utility theory with its later refinements. Psychologists, economists and others in the field are acutely aware of the vast discrepancies between the public's idea of risks and the scientific estimates of the risk assessor. But they decline to attribute them to public ignorance or "irrationality".

Workers in comparative risk perception have emphasized the profile of qualities that go to make up the "meaning" that a hazardous activity has for people. They demonstrate, empirically, that (for example) voluntariness, and familiarity with the risk, its catastrophic potential, its threat to self or to society and the immediacy of its effects are all potent intervening variables. In utility theory, the thrust is towards the complex trade-offs that people make between costs (including risks) and benefits, together with, for example, the "concept of bounded rationality" which explains the changes in the trade-off when the bottom line has to be preserved.

Both of these approaches and more besides are admirably reviewed in Mary Douglas's book. Their limitations are exposed ruthlessly but politely. The author is neither a psychologist nor an economist (despite her erudition in these fields) but an anthropologist. She argues that "...what is needed is to persuade the two sets of analysts that there is a mediating element, *culture*, which is worthy of their combined attention". This is undeniable, though one may be forgiven for mentioning that the book's somewhat single-minded proselytization of an anthropological perspective may itself demonstrate the force of its central argument — there are no stronger cultural influences on the perception of risk than disciplinary affiliations!

In defence of psychology it has to be said that, although it has shamefully neglected the importance of social factors, it is far from unaware of them. It is a parody to suppose that psychologists expect to identify explanatory variables that are determined genetically or in early childhood, and thereafter become part of an enduring personality. What they do seek, however, are broad generalizations, and they certainly recognize that the qualities of familiarity and voluntariness, for example, are culturally determined. Further, the systematic differences in perception between members of different cultural groups (namely the League of Women Voters, upwardly mobile businessmen, students and "experts") were demonstrated by psychologists some years ago. Much of personality is internalized culture.

Once again, a great deal depends on the most profitable level of analysis; there is a sense in which it helps very little to argue that racing drivers, driving-school instructors and housewives all have different perceptions of traffic risks because they are conditioned by different cultural backgrounds. The answer must surely lie in the classification of cultures in terms of the constructions they put upon different hazards — and why. Mary Douglas has made an excellent start in this direction. It has to be added, though, that the exercise will have to "go empirical" if it is to make progress beyond this set of signposts which at present do little more than point clearly and convincingly in the right direction. ☐

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Crusading appeal

Alexandra Dixon & Brian Bertram

Animal Extinctions: What Everyone Should Know. Edited by R.J. Hoage. *Smithsonian Institution Press/Eurospan, London: 1986. Pp.192. Pbk $10.95, £9.45.*
The Last Extinction. Edited by Les Kaufman and Kenneth Mallory. *MIT Press: 1986. Pp.208. $16.95, £12.95.*

THE conservation ethic can be likened to a new religion — not in being based on faith, but in the vigour with which those who are convinced of its necessity strive to persuade others to join them. The target is the unconverted general public, the many uninformed, uninterested or unsocial individuals who persist in contributing directly and indirectly to the destruction of the Earth's natural resources, particularly its living resources. Unlike the religious missionary, conservationists are able to marshal a formidable array of scientific and economic arguments to support the cause. The need is for these arguments to be put across to the general public, and urgently.

These two collections of symposium and lecture papers aim to meet this need. The first, *Animal Extinctions*, will arm the already converted. It contains an assemblage of information on the theory and application of conservation biology, covering such topics as strategies for preserving species in the wild, genetics, sustainable exploitation of wildlife and the economic value of species to mankind. Conservation biologists will have heard much of it before, partly because the symposium was held as long ago as 1982, and the material was not new then. The general public will find its presentation dull and the arguments rather heavy going, but it is useful to have this authoritative textbook available at last.

For the unconverted, *The Last Extinction* is better directed and more effectively evangelical. The editors state in their first paragraph that their aim is to persuade people to rearrange their priorities to avert the threat of mass extinctions, and the contributors set about doing precisely that. The eight public lectures given in Boston, Massachusetts, in 1984 were delivered by a good mixture of representatives from government agencies and the academic, zoo, aquarium and botanical communities in the United States. Using a variety of natural habitats and species to illustrate their points, the authors collectively argue that the looming threat of mass extinctions is *not* a natural event, that it is of fundamental concern to us all and that urgent action is required, to which end they provide clear recommendations and a useful directory of conservation organizations.

While preaching the same overall message, each chapter deals with a distinct issue. "Why the Ark is Sinking" discusses the crucial difference between natural and unnatural extinctions, and why we should care. A chapter on the Amazon illustrates the nitty-gritty realities of local poverty, conflict of interests and ill-considered development, and gives the reader a real idea of the factors involved in conservation on the ground. But conservation is not only about saving exotic species in faraway lands. For instance, few North Americans realize that at least 25 species and sub-species of vertebrate have become extinct in their continent since 1950.

The Last Extinction is compelling reading. It is well written, and attractively presented with a glossy cover and lots of pictures. Absorbing the arguments presented by the authors is quite painless, which is why the book is so effective. The man in the street will enjoy it; he will also learn a lot. He may also join the crusade. We must all hope so. ☐

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Problems of scale in ecology

John Lawton

A Hierarchical Concept of Ecosystems. By R. V. O'Neill, D. L. DeAngelis, J. B. Waide and T. F. H. Allen. *Princeton University Press: 1986. Pp. 253. Hbk $45, £29.70; pbk $14.50, £9.60.*

Ecologists have been slow to wrestle with the importance of spatial and temporal scales in population, community and ecosystem processes. By way of illustration, imagine a coral reef, and the myriads of jewel-like fish that call it home. How important are interspecific interactions between the fish in determining "community structure" — which species live where, when and in what abundance?

Study a single patch of coral a few metres square for a few weeks, and fish may appear to come and go at random: evidence of pattern or structure is hard to find because the spatial scale of observations is too small. Fish communities may, instead, be structured over much larger scales, set by patterns of larval recruitment from the plankton over several kilometres of reef. Or, with a different question in mind, it may be very difficult indeed to link detailed behavioural studies of interactions between fish, over timescales of minutes or hours, to population dynamic processes taking months or years. Finally, the scuba-diver interested in fish behaviour takes the coral for granted as part of the habitat. Yet corals build and die over decades: how are fish behaviour (involving the fastest time- and the smallest spatial-scales) and coral dynamics (with long time- and huge spatial-scales) to be combined into realistic models of the reef ecosystem?

A Hierarchical Concept of Ecosystems tries to address such questions: they may seem obvious, but they have received far too little attention from ecologists. More often than not, field workers arbitrarily select a scale of observations that "seems right", and theoreticians are mute about space and time. But as O'Neill and his colleagues show, these are risky strategies. Theories and observations appropriate for one kind of ecology do not easily translate to larger and longer or smaller and faster spatial and temporal scales. Instead, the natural world can profitably and more correctly be viewed as a multilayered system, hierarchical in space and time. One virtue of this approach is that a number of apparent conflicts are immediately resolved. For example, assemblages of species (communities) may indeed be regarded as more or less separate entities, each with its own rich biology, much as H. A. Gleason suggested over 50 years ago, whilst entire ecosystems may function holistically, fixing energy and cycling nitrogen irrespective of the species involved. Neither point of view is more "correct" than the other. Similarly, populations and communities of organisms may well be equilibrium systems, with population sizes regulated by food or space, embedded in an ecosystem undergoing long-term, very slow changes far from equilibrium. Again the "correct" picture depends upon your perspective and what questions you ask.

The general message of this book is therefore valuable and well put. Its strength lies in the exposition of hierarchy as a way of thinking, in repeatedly drawing attention to the pitfalls of ignoring scale, and in reinterpreting existing data, disagreements and conflicts in the light of the new perspective. In contrast, I found the development of a formal theory of hierarchy, useful for ecologists, more disappointing. The ideas are more qualitative than quantitative, and starting to accumulate their own jargon: it remains to be seen whether ecologists will find the term "holon" useful, and whether holons can be identified, measured and studied in any objective way. Nor do many clear and new predictions emerge from the book. There are some, for example specific predictions about patterns of nutrient loss from stressed laboratory ecosystems ("microcosms"), and much more general and important ideas about overall control of the dynamics of populations, or other ecosystem components, interacting in nested hierarchies of slow and fast timescales. I was disappointed not to find more, but recognize that at this stage in the game more rigorous theory and predictions are hard to come by.

The construction of theoretical models of ecological processes as spatial and temporal hierarchies, and attempts to match, test and refine the models against the real world, will undoubtedly be one of the growth industries in ecology over the next decade. *A Hierarchical Concept of Ecosystems* is a good point of departure for this venture, which could prove to be an exciting and unpredictable journey. ☐

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Even cognitions

Nicholas J. Wade

Odd Perceptions. By Richard L. Gregory. *Methuen, London and New York: 1986. Pp. 230. £16.95, $19.95.*

Editors of journals usually confine their published remarks to matters of policy. Not so Richard Gregory in his editorials in the journal *Perception*, which have appeared over the past 15 years. These essays reflect the ideas that were exciting him as the deadline for publication descended, and a selection of them has now been collected together under the title *Odd Perceptions*.

Many of the pieces have been amended, extended or amalgamated, and the variety of subject matter is vast: from Bacon to exploratories, from eclipses to ESP, from gnomons to von Neumann, from wind-up frogs to Wittgenstein. They are grouped under three headings — amusing, musing and using (alas, there is no fourth musical section!).

Oddly enough, it is not perception that binds the essays together, but the history of science and its associated methods. At times, the history is confined to individual achievements (such as Kepler's law of planetary motion or Turing's approach to machine intelligence), at others it is addressed to a particular problem (mirror reflections or consciousness, for example). All of these issues tend to be reduced to problems of cognition: how ideas about physical processes develop, and how cognitive processes can be modelled.

According to Gregory, these two types of problem are closely related: his thesis, presented in more detail in *Mind in Science* (Weidenfeld & Nicolson, 1981), is that theories of mental processes draw upon the current solutions to technical problems. The methods that work with physical systems should, he considers, be applied to biological ones. Again, oddly enough, very little is said about machine vision, even though the approach he adumbrates has had some of its greatest successes in image interpretation. Gregory's cognitive approach to perception (as outlined in his chapter on illusions) was developed without the benefit of these solutions, and so it would have been instructive to learn whether his theory has been revised accordingly. Perception is conceived of as a process generating and testing hypotheses — much as scientists do with respect to physical phenomena — until a resolution of the problem is found. How the hypotheses are generated is one of the many deep mysteries we are left with; indeed, one of the pleasures of this book is that it does not set out to provide the answers, but to frame appropriate questions, and to place them in historical perspective.

The essays are served with a liberal mixture of autobiographical anecdote, wit and word-play. They stand as a tribute to Richard Gregory's enthusiasm for perception — the process and the *journal*. ☐

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The integrity of the scientific literature

Walter W. Stewart and Ned Feder

A case of admitted scientific fraud has shed new light on the system that ensures the integrity of the scientific literature. Certain lapses from generally accepted standards of research may be more frequent than is commonly believed.

We wish to report the results of an unusual investigation. In May 1981, the colleagues of a respected young scientist were shocked to discover that he was a forger of data. Initially, Dr John Darsee confessed to a single forgery, but it was subsequently found by his colleagues and by three investigating committees that he had fabricated much of the data that formed the basis of his more than 100 publications[1-129] over a period of about three years.

Although many of these publications embody data now acknowledged to be fraudulent, we have scrutinized these publications for internal consistency, accuracy and completeness. Our sample consists of the 109 publications by Darsee with the 47 scientists who were his co-authors, all of whom worked at two leading US medical schools. We also studied the reports of the committees set up to investigate the Darsee affair. What is special about the publications in our sample is that, as a by-product of the extensive investigations into Darsee's work, the circumstances surrounding their publication were known in unusual detail.

Our objective was to discover whether the published scientific reports would throw light on the vigilance of referees, of editors of journals and of Darsee's co-authors in meeting the standards conventionally accepted as necessary in the scientific literature. We have found many of the reports to be flawed in ways that could have been recognized by those concerned with their publication.

We emphasize the distinction between the original forgery and the lapses from standard publication practices we have found in our "sample of convenience"[130]. Other studies will be needed to determine whether or not our results are representative of clinical scientists, of biomedical scientists or of scientists in general.

Background

The circumstances of the Darsee affair have been widely reported[131-141]. In 1978 to 1981 inclusive, he was author or co-author of 18 full-length research papers published in major biomedical journals and of about 100 abstracts, book chapters, letters, reviews and short papers in clinical and experimental cardiology[1-129]. In part because he was so prolific, he was highly regarded first at Emory University School of Medicine and then at Harvard Medical School.

Five months after Darsee's confession, in May 1981, to a single act of data fabrication, it became apparent that there had been more than one such episode. Ultimately, three committees were appointed to investigate. Harvard appointed a committee headed by R.S. Ross, which issued its report[131] in January 1982. The NHLBI (National Heart, Lung, and Blood Institute) appointed a panel headed by H.E. Morgan to investigate the circumstances at Harvard, where most of the research had been supported by the National Institutes of Health (NIH); this report[134] was completed in June 1982. Finally, at Emory, an investigating committee headed by N.C. Moran was formed, and issued its report[132,133] in March 1983.

Shortly after the appearance of the report of the Moran committee, and almost two years after the frauds themselves had come to light, we embarked on our study. We were especially careful to discriminate between the direct effects of Darsee's forgeries, which are not the subject of this report, and the defects in the papers of which he was a co-author. We recognize that some may disagree with some of our decisions as to what constitutes a defect, and with our judgements of the degree to which Darsee's co-authors may be held to have been responsible.

Darsee's publications were in experimental and clinical cardiology, a field of research quite different from our own. For this reason, we have no comments to make on the pointedness or importance of the research described in the publications or about the design of the experiments. No doubt we may also have overlooked some errors in the publications because of our lack of first-hand knowledge of the field. At the same time, we believe that our position as outsiders had the advantage that none of those who published with Darsee was known to us personally or professionally. It may also be an advantage that we have been able to carry out our analysis in the same way that other scientists outside the field of cardiology might approach such a task.

Of Darsee's 47 co-authors, 24 worked at Emory University School of Medicine and 23 at Harvard Medical School. Biographical data were found for 44 of the 47 co-authors. Thirty-nine were apparently MDs, one was a PhD and one held both qualifications; three apparently had no advanced degree. At the time of their involvement with Darsee, about half of the co-authors were 30–40 years old and had received their advanced degrees 5–15 years previously. Approximately equal numbers were senior and junior to this group, the latter including technicians, medical students, resident staff at teaching hospitals and junior faculty members. The senior co-authors included professors and department chairmen. (Darsee himself was 33 in 1981.)

Abundance of errors

Many of the published errors and discrepancies were presumably introduced by Darsee. We nonetheless considered all errors, major and minor, to be of interest because they might reflect on the care with which the co-authors had checked the publications that bore their names, and might reflect indirectly on the care with which they had carried out their share of the research.

All but a few of the 18 research papers[6,24-26,50-53,87-96] (Fig. 1) and many of the abstracts contain errors or discrepancies that can be recognized simply by examining them carefully. We emphasize again that many of the errors and discrepancies are minor, for example, a small discrepancy between a numerical value printed in a table and what is supposed to be the same value printed in the text. Some errors,

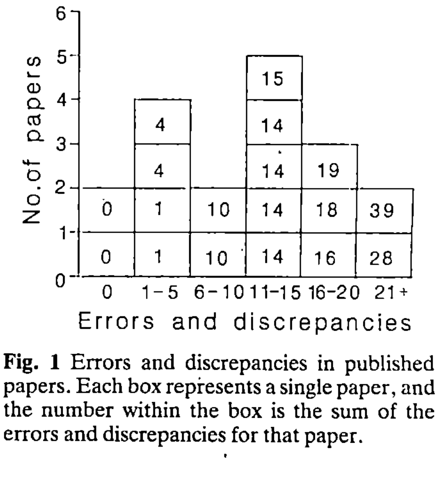

Fig. 1 Errors and discrepancies in published papers. Each box represents a single paper, and the number within the box is the sum of the errors and discrepancies for that paper.

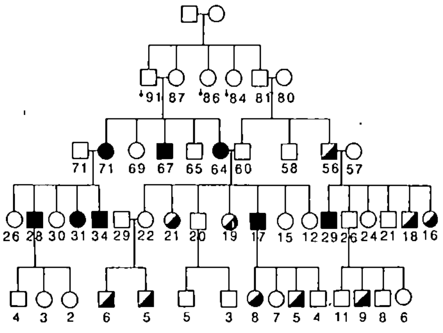

Fig. 2 Pedigree reproduced from ref. 87. The age of each family member at the start of the investigation is given below each symbol. Arrows (second row) denote those who had died before the start of the investigation. Note the 17-year-old male (row 4) with children ages 8,7,5 and 4; at the age of 8 or 9 (probably 8) he impregnated the mother of his oldest child.

however, are so glaring as to offend common sense.

We present a detailed analysis of three sample research papers from which the reader may judge the types of errors we counted. Two papers[87,88] from Emory and one from Harvard[96] are discussed below as examples of our procedure. Two of these[87,96] were eventually retracted wholly[127,142] or in part[143], but one[88] has not been retracted and is still cited as valid[132,133].

The most striking example, in ref. 87, is that in which the authors reported an arresting pedigree depicting a family with a high incidence of an unusual form of heart disease. Inspection of the pedigree (Fig. 2), reveals that a 17-year-old male had 4 children, ages 8,7,5 and 4. (It is unlikely that the father's age is a misprint: it appears in the figure and in two places in the text.) Thus the father was 8 or 9 (probably 8) when he impregnated the mother of his first child and 9 or 10 when he impregnated the mother of his second. This bizarre feature of the pedigree, which perhaps could have raised questions about the validity of the entire paper, was apparently not noticed by co-authors or referees, nor were the following unlikely groupings of ages: his sister, brother and first cousin had their first children at ages 16,15 and 15, and three women in the preceding generation had their last children at ages 41,45 and 52.

The summary of the paper gives the urinary taurine levels of the family members with congestive cardiomyopathy as ranging from 411 to 536 mg taurine per g creatinine, but the text and Table 1 of the same paper give for the same measurement 426 ± 45 mg taurine per g creatinine (mean ± standard deviation). Simple inspection suggests that these two sets of numbers cannot simultaneously be valid. Likewise, the summary of the paper states that the urinary taurine levels of the family members with mitral valve prolapse ranged from 215 to 265 mg taurine per g creatinine, whereas the text and Table 1 give 220 ± 31 mg taurine per g creatinine. These values are central to the thesis of the whole paper. The paper has three numerical discrepancies with ref. 40, and five with refs 19 and 28, abstracts which appeared before the paper and which deal with the same family. All three abstracts of this work indicated that, at the beginning of the study, all 46 members of the kindred were alive, which contradicts Fig. 1 of ref. 87, in which 3 of the 5 oldest are shown as having died before the beginning of the study.

A more subtle example of a major error is illustrated in Fig. 3, taken from a paper[88] also from Emory by six members of our sample. The figure depicts changes in haemodynamic values in five patients at various time intervals after a surgical procedure. Almost all the data in this paper[88] are contained in three figures which cannot be reconciled with each other. The mean and standard deviation for haemodynamic values in the five patients are given in our Fig. 3 (Fig. 1 in the original paper). It is easy to see that many error bars are the same size, and also that mean SVR (systemic vascular resistance) is almost constant at −160 units (the bottom of the graph) in four serial measurements. (Abbreviations are explained in the legend to Table 1.)

These results are especially implausible in the light of data given elsewhere in the paper, where it is shown that SVR and other haemodynamic values for two individual patients vary markedly in the course of time. Moreover, many standard deviations in the figure we reproduce appear on inspection too small to be reconciled with the widely scattered values shown elsewhere in the paper; this is true of SVR at 0.5 h, PVR at 1 h, SWI and SVR at 8 h, and CO at 12 h. Indeed, simple calculations show that it is virtually impossible to reconcile most of the data in the figure we reproduce with the data in the other two figures of the paper.

The introductory section in this paper[88] indicates that the study of five patients was carried out "in four uncomplicated cases of peritoneovenous shunt insertion, and in one subject who developed signs of cardiac failure and pulmonary edema", but the figure legends indicate that two of the five patients developed pulmonary oedema. Specifically, one of the four patients said to be without complications "developed clinical and radiographic signs of pulmonary edema 45 minutes after the shunt was opened" (Fig. 2 legend). The other patient, whose pulmonary oedema was more severe, is described variously as developing this complication "one hour after the shunt was opened" (Fig. 3 legend) and "within a few hours" after the shunt was opened (results section[88]).

Furthermore, the statements that the changes of the mean shown in Fig. 1 after the first half hour were "very small" and that there was "very little change" appear to be contradicted by the actual maximum changes of the mean — a factor of two or more in the change from the value at time zero (corresponding to roughly a 25–60% change in the mean itself) — for three of the five parameters in the figure.

There are obvious inconsistencies between this paper[88] and a preceding abstract[30] describing the same five patients. Eight of ten values for mean or standard deviation given in the two publications are inconsistent (Table 1). This includes most of the numerical data in the abstract. Another comparison (Table 1) shows discrepancies in two out of five standard deviations between one

Table 1 Discrepancies between a paper and two abstracts

	Ref. 88	Ref.30	Refs 20 and 88	Refs 20 and 30
Mean or s.d.	m.±s.d.	m.±s.d.	s.d.	s.d.
Parameter measured:				
CO	2.1±0.8	2.6*±0.8	1.0†	0.9
PCWP	10.0±3.7	12*±2*	4.4	1.8
PVR	−43±33	−64*±24*	30	43‡
SVR	−123±33	−192*±168*	199†	158
SWI	10.6±4.2	11.3±5.8*	4.8†	5.2‡

Ref. 88 is a full-length paper; refs 20 and 30 are abstracts. Discrepancies are indicated between columns 2 and 1 (*), columns 3 and 1 (†), and columns 4 and 2 (‡). Values in column 1 were obtained by measurements of the 0.5 h points in Fig. 1 of ref. 88. The values in column 2 were copied directly from ref. 30, except that for SVR and PVR, the R units[30] have been converted to dyn s cm^{-5} (factor of 80; ref. 144). Columns 3 and 4 contain standard deviations for 5 patients calculated from the means and standard deviations given in ref. 20 for 4 patients and the means given in refs 88 or 30 for 5 patients. The values were derived from calculations based on "population" standard deviations; calculations based on "sample" standard deviations gave similar values and conclusions. When comparing values, allowance was made for rounding of the final digit in refs 20 and 30 and for uncertainties of measurement in ref. 88. Abbreviations: CO, cardiac output; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance; SWI, stroke work index. Units are given in the publications, except as noted above.

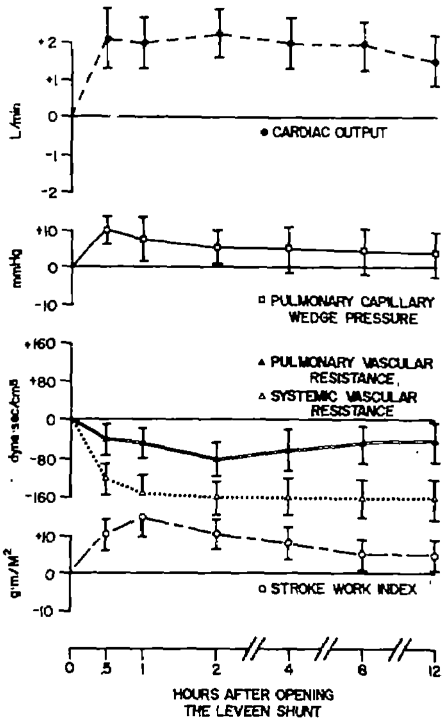

Fig. 3 Reproduction of Fig. 1 from ref. 88. The graph depicts the mean and standard deviation for changes in haemodynamic values of all five patients in the study. Note the remarkable similarity in size of many error bars. Note also the relative constancy of SVR (average value for five patients) at −160 for four serial measurements, despite the variability of SVR (individual values for two patients) in Figs 2 and 3 of ref. 88 (not reproduced here).

abstract[20] and the other[30], and in three of five standard deviations between the abstract[20] and the paper[88]. Thus the two abstracts are inconsistent with each other and with the full-length paper.

In both research papers described above, the major errors are fundamental and call into question the validity of the paper's main conclusions. More typically, however, the errors simply reflect on the care with which the paper had been prepared.

Table 2 Frequency of lapses classified by publication and by co-author

Type of lapse	No. of publications	No. of co-authors
1	28	26
2	42	21
3	7	9
4	13	6
5	7	6
6	1	4
7	8	8
8	1	1
9	1	1
10	2	3

Column 1, type of lapse as defined in Table 5. Column 2, number of publications by co-authors in which there were one or more examples of the lapse in column 1. Column 3, number of co-authors involved in each type of lapse. Some publications and some co-authors were associated with more than a single type of lapse.

The final example was contributed to a major journal by one of the co-authors about two months after Darsee had admitted to him and another co-author that he had forged data in another study. At the time the paper[96] was contributed, these two co-authors were carrying out, privately, a "detailed review and analysis"[131] of the extent of Darsee's forgery in their laboratory. Thus these two scientists were simultaneously publishing a paper containing the results of their collaboration with Darsee and serving in private as investigators into the extent of his forgery of data. One author stated[138] that he had "total confidence" in this particular paper, which he also said[135] had "passed special scrutiny". The Ross Committee, asked to review Darsee's work at Harvard and prepare a statement of record, affirmed the co-authors' view that this publication was accurate.

The paper[96] describes the recovery of heart muscle injured reversibly by a brief experimental occlusion of a coronary artery. One measure of heart muscle function (systolic shortening) is said in the legend to Fig. 3 not to recover fully "until after 7 days of reperfusion". This description is at least unclear, and we regard it as erroneous, since the figure itself and the data in Table 1 show full recovery at or before 7 days.

We present this as an example of an error of very minor significance, but one that we think suggests that the co-authors were not sufficiently careful in examining their own paper before publication. There are small discrepancies between the summary (p. 7152) and the text (p. 7153) with regard to ATP concentration at both 90 min and 72 h of reperfusion. There is a discrepancy between the error bar for ischaemic subendocardium at 15 min of occlusion in Fig. 1A and the corresponding number in the summary and text.

The paper also contains discrepancies with another paper[95] and with an abstract[105], both published previously, and both describing similar or identical studies on similar groups of dogs. The values of maximum negative dp/dt (all between about − 4,000 and − 4,300 mm Hg s^{-1}) for the dog in Fig. 3 of ref. 96 cannot be reconciled with the values (means from − 2,248 to −2,562; standard deviations from 200 to 316) presumably calculated from this dog plus 6 others and listed in Table 2 of ref. 95. Also, the size of the experimental groups for measurement of ATP and creatinine phosphate at 15 min occlusion and 90 min reperfusion is different in the three publications: 14 or 21 dogs (the text is ambiguous) in ref. 95; 34 or 41 in ref. 96; and 36 in ref. 105.

Despite these differences in the numbers of dogs in the experimental groups, the means and standard errors, wherever given, are the same for all three publications. For example, in refs 95 and 96 (Figs 1 and 2, 15 min occlusion and 90 min reperfusion), there are 16 values and 16 error bars; these are identical in the two papers. Also, in the cases where numerical data are given in the text of the three publications, the numbers after rounding are the same. Such a coincidence of means and standard errors from experimental groups of different sizes is extremely implausible. The paper[96] contains additional errors and discrepancies not described here.

Among the 18 research papers, there were as many as 39 errors and discrepancies in a single research paper, with an average of about 12 per paper (Fig. 1). Of the 22 scientists who were co-authors of a research paper, 19 were co-authors of at least one research paper containing 10 or more errors or discrepancies.

The analysis of errors and discrepancies given above is based entirely on our sample of publications in scientific journals. In contrast, some of the analyses that follow depend on information given in the committee reports.

Retention of data

The reports of the investigating committees showed that in almost all cases most of the co-authors had not retained the experimental or clinical data on which the publications were based. This was true of 7 of the 8 Harvard papers, which involved experiments on dogs, and of 8 of the 10 papers from Emory, 8 of which involved human subjects. [These figures appear mostly attributable to the failure to retain data by Darsee, the chief source of experimental data — Editor, *Nature*.] In the latter case, there were 7 papers for which some of the co-authors had retained neither names nor other identifying information for the human subjects. For the purposes of the present report we have disregarded all papers not involving human subjects except for the cases discussed below.

Of 14 who were co-authors of papers (all from Emory) involving human subjects, at least nine failed to retain the list of subjects (Table 2). One paper[25] is particularly interesting: a report on the incidence of mitral valve prolapse in presumably healthy young men. The paper is considered valid[133] and is heavily cited[145]. The co-authors "maintain that the data in the paper are valid although none of them has a copy of the original research results. They state that they did the study and oversaw the writing of the paper"[133]. Although Darsee was first author of the paper, the Moran report provides no information on his role in the project and, in particular, on his opportunities for altering or forging the data; possibly this information is contained in the confidential appendices to the report. Thus the only available support for the validity of this paper is based on a statement attributed by the Moran Committee to the co-authors, given without supporting data or details.

Involvement in research

In considering involvement, we confined our attention to the 18 research papers. We defined "direct involvement" to mean actual participation in the acquisition and interpretation of data, or the making of an intellectual contribution which was essential to the research. We disregarded a person's rank in the laboratory hierarchy in judging whether an author was directly involved.

Our evidence for the extent of an author's involvement came from the reports of the investigating committees. For example, according to a committee report[132], one co-author's role was to encourage Darsee and provide some of the grant support for the research. In another committee report[134] a senior scientist is described as having other duties not allowing him sufficient time for direct participation in the research laboratory and also as being seldom in the laboratory and having no direct supervisory role. In both these cases we considered that the co-authors had not met our criteria for direct involvement, in the sense of direct participation in experimental work. Supervisory functions may be exercised with a greater or lesser degree of remoteness, and we acknowledge that the evidence available is limited and that our judgements on this point are necessarily subjective.

We consider those not directly involved in the research to be "honorary" authors. Honorary authorship was a common occurrence, according to our analysis. Of 18 papers, 13 had at least one author judged by us to be honorary. There was a total of 49 authorships (that is, occurrences of an author's name on one of the 18 research papers); in 16 authorships (33% of the total), involving 6 of the 47 co-authors, the author was judged to have little or no direct involvement (Table 2). Those who were honorary authors at least once also published significantly more papers than those who were never honorary authors (3.33 ± 1.63 versus 0.71 ± 1.42, mean ± standard deviation; $P < 0.01$ by Student's two-tail t-test). Their higher rate of publication compared with co-authors who were not honorary appeared to depend entirely on their honorary papers: the difference disappeared when an author's honorary papers were not counted (0.67 ± 0.82 versus 0.71 ± 1.42). The 13 papers with at least one honorary author had more errors and discrepancies than the 5 without an honorary author (15.1 ± 10.2 versus 5.0 ± 6.6; P= 0.067).

Table 3 No. of dogs for which results were shared

	Ref. 53	Ref. 90	Ref. 91	Ref. 92	Ref. 94
Ref. 53	**33**	8	12	0	9
Ref. 90	8	**22**	9	0	11
Ref. 91	12	9	**76**	6	7
Ref. 92	0	0	6	**48**	0
Ref. 94	9	11	7	0	**44**

The boldface figures (on diagonal) give the total numbers of dogs used in each study. The remaining entries give the numbers of dogs for which experimental data were shared between pairs of studies. Although not shown in this table, data for the same 2 dogs were used in 4 studies[53,90,91,94]. Reference 134 was the source of this table, which in turn was based on information provided by one of the senior co-authors at Harvard.

Incomplete statements

In many ways the most striking of our findings was the discovery that certain research papers (7 of 18) embody statements and data in a manner that impedes the reader in forming an accurate reconstruction of the way in which experiments were actually carried out. The circumstances were these.

Five papers[53,90-92,94] reported studies done with dogs on the effects of experimental coronary artery occlusion on the heart and on recovery from the effects of occlusion with or without drugs. In these papers the assignment of dogs to experimental and control groups is described as formally randomized, as with the statement[90], "Twenty-five minutes after occlusion of the LAD, the remaining dogs were randomized to treated or control group on the basis of a random odd or even number generated by a computer program."

During the subsequent investigation by the NHLBI panel, however, the co-authors acknowledged that some control data had been reused[134] — in other words, that the control dogs actually included some historical controls, a use which had not been mentioned in the published papers. (Historical controls are animals that have been used as controls in a previous study[130]. The same data recorded in the earlier study are then used again as control data in the later study. The number of dogs for which data were reused is shown in Table 3.)

One co-author of three of the five papers subsequently stated[146]: "In order to economize animal usage, as well as professional and technical time, control animals which had previously been randomized were sometimes used to provide data for more than a single experiment . . . we saw no reason not to allow him [Darsee] to use his randomized controls in more than a single experiment." Another scientist, the co-author of all five papers, acknowledged the reuse of data, but stated[147] that Darsee had misled him and the other co-author about the number of dogs for which data were reused and about the number of experiments in which the data for any one dog were reused.

Reuse of data was approved by one of these co-authors in at least one paper not involving Darsee (mentioned in ref. 134), and both co-authors have subsequently defended the practice. One co-author, commenting on a study not involving Darsee that was carried out in his laboratory, acknowledged[147] that control data were reused and that the reuse was not described on publication: "Although the use [of some control data from another study] was not specified in the text [of a study not involving Darsee], the use did not bias the results of the study in any way". The other co-author defended the sharing of control results said to be randomized by stating[146] that "the practice of not specifying in the Methods Section that a control dog had been used in more than one study, while certainly not ideal, is common," and also that "the practice of utilizing controls in more than a single experiment is not uncommon and, *although it should be*, it is not always spelled out in published papers" (emphasis added).

In a series of experiments in which the objective is to study the effects of various interventions on animals subjected to a certain procedure, in this case arterial occlusion, the ideal is that described in this group of five papers: all animals would be subjected to arterial occlusion, and afterwards assigned randomly either to the control group or to one or other of the experimental groups.

Although the use of historical controls may have several practical advantages, not least those of economizing in the use of animals, this practice is clearly less satisfactory than the ideal; for example, the experimenter's foreknowledge could systematically affect the outcome of experiments. The fact that Darsee may not have selected historical controls at random, as required by the laboratory's policy described to the NHLBI panel, is irrelevant to our criticism, which is that these papers leave the reader with the impression that control animals were selected by a randomization procedure that most readers, we judge, would consider superior to that actually used.

We emphasize that we do not criticize the use of historical controls, which may or may not have been appropriate, but merely the misleading published description. Thus the use of historical controls is reconcilable with the statement in the paper quoted above that "after occlusion . . . the remaining dogs" were randomly assigned to different groups only if "remaining dogs" is understood to refer to a number of historical controls.

It would appear from the subsequent statements of the two co-authors that they knew of and authorized the use of historical controls in the laboratory, as well as the failure to describe that use on publication. The described randomization procedure in any study was supposed to apply to all dogs in that study, but in fact did not apply to the historical controls when their data were reused. We conclude that the papers contained an incomplete and misleading description of experimental procedures.

Furthermore, the experiments reported in the five papers included procedures, such as arterial catheterization and administration of certain drugs or dyes, that differ from one study to another (Table 4). As a result, most of the historical controls had been subjected to procedures in an earlier study that were different from those described for the later studies. Thus the published descriptions of these later procedures, which are described explicitly and in detail as applying to all dogs, do not apply in some respects to the historical controls.

One author, writing to the NIH about the sharing of results, stated[146] that "the experimental conditions were similar" for the studies in question. The other referred[147] to studies (not involving Darsee) in which "experimental conditions are the same"; he has also indicated in correspondence with us that the procedures described in the five papers were similar enough in some cases so that the use of historical controls was justified. Differences in experimental conditions are summarized in Table 4.

Some might consider that in most cases shown in Table 4, the data from dogs in one study would be unsuitable as reused data for another study, but the facts enabling readers to form a judgment on this point were not given in any of the five papers. We believe that the descriptions of experimental procedures did not apply to the reused data obtained in previous studies and that these descriptions were therefore misleading.

A final point may be related to reuse of data: one of the five papers states[91] that "all dogs [were subjected to an experimental procedure, which is described]. Ventricular fibrillation or standstill was then induced by a barbiturate overdose, and the hearts were excised rapidly and placed on ice." Yet the co-authors have said (cited in ref. 134) that some of the same dogs were subsequently used for the 4-hour and 6-hour occlusions in other experiments. As the NHLBI panel report politely observes[134], "This would appear to be impossible". We have assumed that the two co-authors in our sample were unaware of this anomaly at the time of publication.

The misleading statements noted above appeared in five papers co-authored by two members of our sample. In addition to these five papers, there were two others (refs 95 and 96; see next section) containing statements that were imprecise or misleading. In total, there were 18 instances of statements made by 6 co-authors whose effect would have been, in our opinion, to mislead the reader.

Unacknowledged republication

We were surprised to discover several instances in which the same data were republished without adequate reference to that circumstance. The most striking example was a paper[95] containing data later published almost in their entirety as a major part of a subsequent paper[96] which appeared in a different journal. Although the second paper referred to the first by means of an index number in the text, there is no explicit statement in the second paper that the new data are a continuation of an experiment already published.

The subject matter of both papers concerns the recovery of heart muscle injured by temporary surgical occlusion of a coronary artery in dogs, described by the authors as a model with "important clinical implications for patients" with certain kinds of heart disease. The first paper states that "Seven dogs were also subjected to 15 min of LAD occlusion followed by 3 days of reperfusion", which is not false but which does not reveal that the same 7 dogs were actually reperfused for 14 days. The references in the second paper to the first would not suggest to those who were not familiar with the first that the research about to be described was a continuation of an experiment already published. In the second paper's opening paragraph, which is reproduced overleaf, the first paper is cited twice, but neither citation of the first paper provides a hint that some of the same data are being republished and that some of the same animals were used.

On neither paper was Darsee the first or last author, and it seems that the co-authors could have known of the statements that were imprecisely misleading. For the journal to which ref. 96 was submitted (*Proceedings of the National Academy of Sciences*), a contributing author customarily by-passes the refereeing system, which might have discovered the unacknowledged republication of data; instead the contributing author assumes "responsibility for the propriety and scientific standards"[148] of the manuscript he submits.

Table 4 Differences between experimental procedures in five papers

	Ref.53	Ref.90	Ref. 92	Ref. 94	Ref. 91
(1) Coronary artery occlusion (time)	2 – 6 h	6 h	1 or 3 h	4 – 6 h	1 min – 2h
(2) Randomization before/after coronary artery occlusion	After	After	Before	After	Before
(3) Time from removal of coronary artery clamp to end	~1 min – 4h	Not removed	5 or 72 h	Not removed or 2h	6 h
(4) Antiarrhythmic agent:	Lido	Lido	Lido	Proc'de	None
(i) Initial bolus inj., mg per kg	1.5	1.5	1.5	3	None
(ii) Further intermittent inj.	Yes	No(?)	No(?)	No(?)	No
(iii) Continuous infusion	No	No	No	Yes	No
(5) Dye administered initially	Fln	MeB,GV	Fln,GV	Fln	Fln,GV
(6) Dye injected terminally	TS,MeB	MeB	MeB	MonB	MonB
(7) Drug or NaCl soln, ml per kg	0(?)	1	1	2	0(?)
(8) Catheter in aorta/left carotid	Carotid	Carotid	Carotid	Aorta	Carotid
(9) A set of multiple procedures, (a) – (e), listed below	No	No	Yes	No	Yes

There are substantial differences in experimental procedures for at least nine of the ten pairs of papers. The procedures in item 9 include: (a) insertion of micromanometer through stab wound in ventricular apex, (b) application of coronary cuff, (c) repeated brief reocclusion of coronary artery, (d) implantation of crystal transducers in myocardium, and (e) delay in examination of heart after death (for flowmeter calibration). Abbreviations: *Lido*, *lidocaine*; *Proc'de*, *procainamide*; Fln, fluorescein (injection); MeB, methylene blue (injection); GV, gentian violet (topical); TS, thioflavine S; MonB, monastral blue. Information in the table comes from the papers themselves[53,90–92,94]; see also ref. 134, appendix 5.

Opening paragraph of ref. 96:

Previous studies from this laboratory have shown that brief (15 min) coronary occlusions followed by reperfusion do not cause necrosis but do result in prolonged abnormalities in myocardial biochemistry, function, and ultrastructure (1 – 3). At 72 h after release of a 15-min coronary occlusion, the ATP concentration in reperfused previously ischemic myocardium was significantly reduced (by 22% of normal), the percentage of systolic shortening of reperfused left ventricular segments was reduced (by 42% of normal), and ultrastructural abnormalities were present, despite the absence of necrosis (1, 3). However, it was not known whether these changes ever were reversed to normal after longer periods of reperfusion and, if so, what the time course of the recovery is. Recently there has been renewed interest in the treatment of acute myocardial infarction by means of coronary reperfusion, either by injecting fibrinolytic agents directly into the obstructed coronary artery or by coronary revascularization (4, 5).

This is the opening paragraph, printed here verbatim, of a research paper[96], discussed in the text. The two occurrences of reference 3 in the paragraph above are the only references in this paper[96] to the authors' own previous study[95] (see text).

A common example of republication involves abstracts of research submitted to scientific societies. Of the 88 abstracts, only 47 were published only once; all the rest involve duplicate or triplicate publication. This practice is apparently followed in many fields of biomedical science, where an abstract may be prepared for one meeting of a society and then submitted, perhaps in an amended form, to a second meeting. We nevertheless suspect that the extent of this practice may not be widely appreciated. We discuss four cases below but have not included abstract data in our tables.

We note that there were four pairs of Darsee abstracts in which the texts were nearly identical, but whose titles had apparently been changed. For example, an abstract[77] entitled "Can tetrazolium stains reliably identify myocardial infarction prior to definite histologic necrosis?" was also presented[73] under the title "Early pathologic detection of acute myocardial infarction". Sometimes the alteration consists of the substitution of synonyms[80,72]: "Persistent myocardial abnormalities following brief periods of temporary coronary occlusion not associated with necrosis" also appears as "Prolonged metabolic, functional, and ultrastructural abnormalities following transient myocardial ischemia without infarction".

In each pair the authors are the same, and the text and numerical values are identical through most of the abstract. The publication dates were generally within about a month of one another, as were the scheduled presentation dates. Although it could be argued that it may be necessary to tailor the titles of certain abstracts to make them acceptable for oral presentation before the different societies, we consider that the change in title has the effect of making the pair of abstracts, if listed only by title, appear different to the reader, whereas in fact the contents are almost the same.

There were 5 instances of unacknowledged republication of data. They appeared in publications, two from Emory and seven from Harvard, having a total of 8 co-authors (Table 2).

Lapses from standards

We were surprised to find that there is apparently no generally accepted written code of conduct for scientific research[149-159]. We think that there is nonetheless an unwritten code of which scientists are aware and to which they profess adherence. We have based the analysis that follows on standards that we believe are generally accepted — accepted in the sense that they are generally understood, not necessarily in the sense that they are always complied with.

In Table 5 we attempt to place in ten categories the departures from generally accepted standards that we observed in our sample; other categories would probably be more appropriate for other studies. We found it useful to divide our categories into two groups, A and B, with those of group A explicable simply by carelessness or excessive haste, while those of group B appeared to be more serious in some sense. We do not mean to suggest that certain departures from accepted practice which appear in group B may not be due solely to carelessness and haste; undoubtedly this is so in some cases. Furthermore, we recognize that the distinction between type A and type B is necessarily subjective and that other observers might classify the lapses we have found differently.

We emphasize that the instances on which Fig. 4 is based were supported by evidence we considered compelling, but not necessarily conclusive in each case. We acknowledge that our categories must be subjective.

Frequency of lapses

Of the 47 co-authors in our sample, 31 (66%) were authors of publications containing lapses of type A — a result that is the same whether or not honorary authorships are included. Furthermore, 13 (28%) were involved in type B lapses. Only 12 of the 47 were found not to have been involved in lapses of either type. Most of the 12 were authors of abstracts only; just 2 of the 12 were co-authors of a research paper.

Some scientists apparently do not regard abstracts as publications, even though they are archived and cited and are sometimes used to establish priority[160]. If the 88 abstracts and 3 chapters are excluded from consideration, the frequency of type A and B lapses is about the same or higher. Of the 22 scientists who were co-authors of the 18 research papers, 21 (95%) were involved with papers containing lapses of type A, and 6 (27%) were involved in lapses of type B.

Discussion

It is obvious that the lapses from generally accepted standards of research described here have at least the potential for interfering with the accuracy of the scientific literature or for harming the scientific enterprise in some other way. We also think that the instances described above represent departures from the generally accepted standards of scientific research. We recognize that the decision as to what constitutes a departure from generally accepted standards is necessarily subjective. Presumably some scientists have views different from those expressed here. We regard it as a matter of the greatest importance that these differences be discussed publicly.

Table 5 Lapses from generally accepted standards

Type A

(1) Presence of errors (usually in numerical values).
(2) Inconsistency with a research group's previously published data.
(3) Failure to retain names or identifying numbers for human subjects included in a published paper.
(4) Honorary authorship (see text).

Type B

(5) Statements that are misleading.
(6) Misleading citations of a previous paper containing data being republished, so that the true relationship between the two papers is obscured.
(7) Publication of very similar abstracts under very different titles.
(8) Failure to utilise unique knowledge that makes possible the recognition of certain serious errors in published work — errors not discoverable by anyone else.
(9) Failure to acknowledge the source of a substantial amount of research data received from someone else.
(10) Failure to take appropriate action after receipt of a complaint, later shown to be well founded, that a colleague may be involved in questionable data collection.

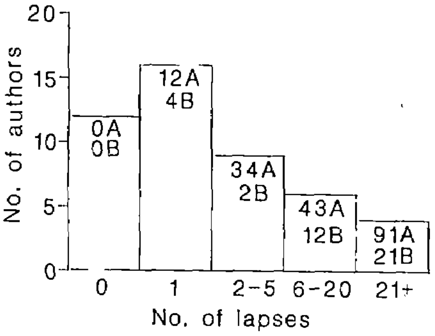

Fig. 4 Authors and their lapses. The histogram gives the number of authors in our sample having lapses within the specified range. The numbers give the total of type A and type B lapses for all authors in that range.

The abundance of errors that we observed in our sample obviously interferes with the accuracy of the scientific literature. Every scientist has, at a minimum, an obligation to ensure that what is published under his name is accurate.

There is a notable absence of explicit published standards for the retention of data. We believe that it would be generally accepted that data should remain accessible for as long as questions and criticisms are likely to be raised about what has been published. For most of the papers in our sample, data were not available. The data we give in Table 4 use only those cases where data involving human subjects had not been retained. We suggest that a reasonable minimum requirement is that each author of a paper involving human subjects should retain at least a list of the subjects' names or the hospital's identification numbers of patients. This information would permit access to hospital records and thus in many cases allow reconstruction of data in the paper.

The reader may ask: What harm is done by honorary authorship? Indeed some of our colleagues have argued that the custom of routinely placing the name of a senior scientist, usually the head of the laboratory, on a paper — regardless of his contribution — is widely followed and does no harm. We disagree, as have others[159,161-166]: honorary authorships falsify the assignment of responsibility for published research and increase the likelihood that inaccurate data will be published. The honorary author is in a poor position to judge the validity of the work, yet he often lends a prestige that may lull other co-authors, the reviewers or the readers into uncritical and inappropriate acceptance.

Similarly, the harm done by unacknowledged republication of data may not be immediately obvious. But it disguises the true relationship between published papers and yields no benefits other than for the authors.

In some ways the most disturbing of our findings is the discovery that certain of the publications (7 out of 18) embody statements and data in a manner that impedes the reader in forming an accurate reconstruction of the way in which experiments were carried out.

Representativeness

The evidence presented here indicates a surprisingly high frequency of lapses from accepted standards. One possible explanation is the association of the co-authors of the publications in our sample with Darsee: he may have induced them to behave in ways not typical of them. A second possibility is that the co-authors are not representative of their field. A third possibility is that lapses from accepted standards may be unusually common in the specialty in which these scientists worked, in clinical research generally or in research carried out at medical schools or by physicians. (The co-authors of the publications in our sample were mainly physicians, and the research was carried out exclusively in medical schools.) A fourth and disturbing possibility is that lapses from accepted standards may be more common among biomedical scientists than is currently appreciated.

Other studies will be needed to determine whether our results are representative of other groups of scientists.

Causes

Extraordinary emphasis is commonly placed on the sheer quantity of publications[158,165–179]. The director of one of the world's leading research institutions (but not an institution with which Darsee was ever affiliated) sent a signed, official memorandum to junior colleagues proposing incentives for publication: "There is no demand that these be literary masterpieces in first line journals; journeyman works for publication in second, third or fourth line archival publications will be quite satisfactory. Upon proper completion and submission of [two] manuscripts, [a technician]'s appointment will be extended to April 1, 1984. During that time it is expected that an additional manuscript on [subject] will be completed and submitted. If so, the period of employment will be extended an additional three months and again an additional manuscript on [subject] is an anticipated result of the extended employment." On another occasion a director of research said in all seriousness, "No research that results in a single paper is worthwhile."

Those promoting Darsee or recommending his promotions were well aware that his publications were unusually numerous[146]. But apparently none of these supporters, who were also his co-authors, made the effort that would have shown that many of the papers had serious flaws. How often does this occur?

Other causes of lapses from accepted standards may be the extreme competition for research grants, large research teams in which the leader is not directly involved in the research and the absence of an accepted code of conduct for scientific research.

In this paper, we have deliberately avoided a discussion of the cases of acknowledged fraud which have come to light in recent years, concentrating instead on defects of the scientific literature generally regarded in the scientific community as regrettable but less serious. Outright fraud, which might conveniently be called a lapse of type C in our nomenclature, is presumably rare. May not the scientific community be over-sanguine in attaching less importance to lapses such as we have found, which are generally unremarked but which debase the scientific literature?

Testing the system

The results of our study naturally raise the question: what fraction of papers in the biomedical sciences are not supported by primary data at the time of publication? The question cannot be answered with the papers in our sample, since most are known to be partial or complete forgeries and thus are atypical.

We suggest a study to answer the question: close examination of a random sample of published papers. Probability of selection could be uniform or could be weighted by a variable such as number of authors or cost of research. Papers would be examined by either of two methods: careful examination of the paper itself for errors and discrepancies by appropriate experts following an established procedure ("external audit") or examination of the primary data on which the paper is based, which would of course require access to laboratory records ("internal audit"). The advantages of the external audit are its non-invasive character and presumably lower cost; the advantage of the internal audit is its completeness.

What would this cost? This depends on the precision required and on the actual fraction of defective papers. We assume for the sake of illustration that an external audit could be done for about 3% of the cost of the research on which a paper is based, and an internal audit for about 10%. If the actual fraction of papers that could not be substantiated were as high as 15% and if the answer were required with a standard error of 7%, about 25 papers would have to be examined, which in the case of internal audits would altogether cost only about 2.5 times as much as one average paper. If, on the other hand, auditing 100 papers failed to disclose a single paper that could not be substantiated, this would show with $P = 0.05$ that the actual fraction of papers unsupported by primary data at the time of publication is less than 3%.

In addition to the financial costs of examining the practices of scientists, there are other costs which may be more serious in the long run. Examination of scientific practices could cause unwarranted harm to individual scientists. Systematic examination of scientific practices might even weaken the fabric of trust that is essential to the functioning of science. Of all human endeavours, science is one of the most successful — prodigious in benefits, low in cost. But science, vulnerable to abuse from within by its practitioners, is perhaps even more vulnerable to harm by regulation, and at some point the cost of further regulation will outweigh the benefits.

Scientists have to an unusual degree been entrusted with the regulation of their own professional activities. Self-regulation is a privilege that must be exercised vigorously and wisely, or it may be lost. ☐

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● Some editorial changes have been made in this manuscript without the consent of the authors. A reply from Braunwald follows on page 215.

On analysing scientific fraud

from Dr Eugene Braunwald, Harvard Medical School

ANYONE who has been a victim of scientific fraud or who has had close contact with such an episode must be sobered by the event and must ask how the fraud could have been prevented or detected earlier. The costs are incalculable, not only for the persons and institutions involved, but for science as a whole. An objective analysis of research practices which offered some insight into the nature of scientific fraud and the manner in which it could be prevented or detected would be an important contribution to the public discussion of this critical issue.

I agree that many of the issues Stewart and Feder raise are important and deserve attention and thoughtful analysis. When specific incidents of scientific misconduct are discussed, however, it is essential that the facts be dealt with accurately and objectively. Unfortunately, Stewart and Feder do not provide such a discussion of the events surrounding the Darsee case insofar as they occurred at the Cardiac Research Laboratory at Harvard and the Brigham and Women's Hospital.

As the paper has been widely circulated in many different versions and its contents have been released to the lay press before publication, I am pleased to have the opportunity to reply to the paper with respect to the work done by the 23 coauthors at Harvard.

John Darseee committed serious fraud in at least three (not two) universities over a period of at least 12 years. The premise implicit in Stewart and Feder's paper is that it is possible to separate clearly *this* fraud committed by Darsee from the professional behaviour of his coauthors. In fact, the paper repeatedly and unfairly connects Darsee's fraud at Harvard to his coauthors there through a process of guilt by association. As a result, many of Stewart and Feder's allegations are misattributions of Darsee's wrong doing to his coauthors. Other allegations involving the papers from Harvard — all of which were retracted in 1982 — are insubstantial or simply wrong and have nothing to do with scientific misconduct.

Allegations of errors

Stewart and Feder assert that there were errors in the Harvard papers although the specifics and location of those purported errors in the papers have not been provided despite numerous requests. This assertion is highly dubious as the few alleged errors which Stewart and Feder actually identify prove, upon careful scrutiny, not to be errors at all or to be wholly trivial errors which most scientists would agree do not rise to the level of a "lapse" in research standards.

Stewart and Feder discuss one Harvard paper, Paper 96, in which they purport to have identified five errors. The first involves a supposed semantic inconsistency concerning when systolic shortening of the previously ischaemic heart returned to normal. The legend to a figure in Paper 96 stated that recovery of systolic shortening did not occur until *after* 7 days, but Stewart and Feder claim that the figure and a table reflected recovery *at* 7 days. Paper 96 makes clear that the data presented in the figure and the table were obtained at the end of 7 days and the phrase "after 7 days" should not mislead anyone. If this represents an error in phrasing at all, I agree with Stewart and Feder that it is of "very minor significance".

Equally insignificant are two claimed discrepancies between values reported in the summary and text of Paper 96, which Stewart and Feder concede are "small". Whether these small differences resulted from the different methods by which these figures could be calculated (on an experiment-by-experiment basis versus a group basis) or from typographical errors, they did not affect the findings of Paper 96 and would not mislead any reader. The general understanding of scientific fraud is hardly advanced by a discussion which hinges upon typographical errors and similar quibblings.

Stewart and Feder also point to a supposed discrepancy between the value of negative d*p*/d*t* for one dog in Paper 96 and for six dogs in Table 2 of Paper 95, which had been submitted for publication six months earlier. These alleged errors can be identified only with hindsight, for there is nothing inherently suspicuous about the error bars or the d*p*/d*t* values. Although these may ultimately be inaccurate as a result of Darsee's fraud, it was not a questionable practice for the Harvard coauthors *at the time* to have accepted Darsee's calculations. Any other course would require coauthors of each paper to recalculate individually every value reported, denying researchers the considerable benefits of a reasonable division of labour. Indeed, in the type of experiment reported, although the absolute values of the cardiovascular variables may differ considerably among different animals; they tend to change in a similar manner over time, and it should not be surprising that the error bars are similar.

Fourthly, the purported discrepancy between the number of experiments in the two papers and the similarity of the results relates to the fact that the number of animals studied at various times differed. The results in those animals reperfused for the shortest period were similar in the two papers. Smaller numbers were reperfused for longer periods. Stewart and Feder's suggestion that those values and error bars could not be correct is unfounded. At most, there was ambiguity about which dogs provided biochemical data at different times. This is not a "lapse of standard".

Lastly, Stewart and Feder claim an inconsistency between an error bar in a figure and the numerical values presented in the text and summary. That and other figures in Paper 96 reflect numbers in the text within the technical limitations of artwork in scientific papers.

Retention of data

I certainly agree with Stewart and Feder about the need to retain research data, but they convey a totally inaccurate picture about this practice in the Harvard laboratory because they fail to mention that the NHLBI panel expressly found that only Darsee failed to retain primary data, and that the other Harvard researchers did retain original records. The NHLBI report stated, "With the exception of Dr Darsee, recent trainees [in the Harvard laboratory] have retained original records in customary and acceptable fashion". The laboratory policy on retention of data was clear; Darsee's failure to retain data was in direct contravention of the policy. Stewart and Feder would no doubt agree that it is proper practice in describing results to provide all of the relevant information. They did not have direct information about retention of data; the NHLBI Panel did. It is unfair to criticize the Harvard coauthors about this issue while withholding from the reader relevant information obtained by the panel.

Involvement in research

I fully agree with Stewart and Feder's general conclusion about the corrosive influence of "honorary coauthorship", but, as they admit, their assessment of this issue is based on subjective inferences drawn from "limited" evidence. Had they had access to all of the relevant facts, I believe they would have known that there were no honorary coauthors of Darsee's Harvard papers. I developed the overall research strategy — a continuation of a research programme begun in 1968, wrote the grant proposals which supported much of the work, participated actively in the design of the protocols, reviewed the results on a frequent, usually biweekly basis and participated actively in the interpretation of the data. This amply satisfies stringent requirements for coauthorship. Indeed, failure to designate a collaborator with this level of involvement as a coauthor would be misleading. (Had I not been included as a coauthor, Stewart and Feder would have been justified in criticizing me for not shouldering my share of the responsibility.) How is the cause of science served by making such unfair accusations?

Randomization

Stewart and Feder's discussion of the manner in which control dogs were selected is predicated on a misunderstanding. The laboratory had a policy for randomizing control dogs, as follows: when a series of interventions were studied on myocardial infarct size in dogs subjected to coronary occlusion, a comparison was made between infarct size in treated and control dogs. The latter were selected in the following manner: with the first intervention, all control dogs were selected at random and were studied *de novo* and contemporaneously with the treated dogs. With subsequent interventions, when the research fellow was the same and the experimental conditions similar, some control dogs were studied *de novo*, that is, contemporaneously with the treated dogs, whereas the remainder were selected, again at random, from among the controls which had been obtained at random by the same research fellow a short time before.

This practice saved unnecessary sacrifice of animals, but allowed each intervention to be compared to a valid control group. As we advised the NHLBI panel in 1982, it would have been preferable to present this policy explicitly in the papers, but this would not have influenced the results or conclusions. Like all experimental procedures, this ultimately relied on the honesty and integrity of the individual researchers. What did influence the results is that Darsee violated this policy.

Deeply troubling is that Stewart and Feder quote selectively my statements concerning the laboratory's policy of using control dogs. By specifically excluding from the quotation references to key aspects of laboratory policy, they permit the reader to be misled that the Harvard coauthors knew and sanctioned Darsee's dishonest practices in this regard.

Republication

Stewart and Feder allege four instances of "unacknowledged republication" of data from Harvard, one involving two research papers. The first described successful experiments on 21 dogs which were studied 90 minutes and 72 hours following reperfusion after brief coronary occlusion; 14 of these 21 dogs were sacrificed 72 hours after reperfusion and heart tissue was analysed for histology, biochemistry and electron microscopy with the results reported in this paper. Physiological data obtained 72 hours after reperfusion in seven dogs were also reported in this paper; these seven dogs were not sacrificed. It was deemed necessary to apprise the scientific community of the findings, which had important clinical implications. Therefore, we decided to publish these results as the work continued and additional data became available.

The second paper included substantial new information, including the results of successful experiments on 20 additional dogs. That paper reported biochemical, electronmicroscopic and histochemical data on these 20 additional dogs, which were studied either 7 or 14 days following reperfusion. The second paper also reported for the first time: (1) physiological data obtained 7 and 14 days following reperfusion in the 7 dogs for which data obtained 72 hours after reperfusion had been described in the first paper and which were not sacrificed and (2) histochemical data on dogs sacrificed at 72 hours. The second paper recapitulates the earlier findings, making express reference to the first paper twice in the opening paragraph. Would we have done this if we had wished to conceal republication?

Stewart and Feder suggest that there were lapses in standards in the submission of certain Harvard abstracts. However, these abstracts were prepared in strict conformity with the rules of the sponsoring societies. None was submitted for duplicate presentation at national meetings. To suggest that there is something improper in revising an abstract to enhance its quality and clarity is baffling. For example, Abstract 70 was submitted but not accepted for presentation to the American Federation for Clinical Research. After the abstract was revised to improve its clarity and focus, it was accepted as Abstract 78 for presentation seven months later at the scientific session of the American Heart Association. Stewart and Feder's contention that the Harvard coauthors somehow engaged in a questionable practice when they revised a previously rejected abstract before resubmitting that work flies in the face of commonly accepted practice.

Conclusions

Just as it is incumbent on scientists to be meticulous about the manner in which they carry out their work, so it is incumbent on analysts of the conduct of science to be meticulous about their activities. Just as it is damaging to science to make exaggerated claims not supported by data, so is it damaging to science to make exaggerated criticisms and allegations about the conduct of science which are not fully supported by facts. Just as scientists must report the results of all relevant experiments and not select just those which support their conclusions while deleting those which do not, so should analysts of science adhere to the same rules.

Despite my differences with Stewart and Feder's analysis regarding Darsee's work at Harvard, the larger problem which they address, albeit obliquely, namely that of fraud in science, is regrettably still with us and must not be swept under the rug. When we discovered and ultimately exposed all of Darsee's misconduct we were staggered at its extent. Nothing in my more than 25 years in research prepared me to deal with it. In retrospect, there are aspects of the matter that could have been handled better. Perhaps one positive outcome of the unfortunate Darsee affair is that it has helped to focus attention on fraud in science, in part prompting both research institutions and granting agencies to develop more formal rules and procedures for dealing with suspected fraud.

I have thought much about fraud in science during the past five and a half years. Although I have not found any simple, new solutions, a number of issues are clearer to me now.

First, it must be understood by all that to be a scientist is a privilege and that society invests a special trust in all scientists. Whenever that trust is abused it diminishes all scientists. Second, because the costs of serious fraud are so high, we must be more vigilant and more sceptical, always alert to the possibility, however remote, that data we have been shown have been misrepresented. Third, in awarding promotions and grants, undue weight is sometimes given to the quantity as opposed to the quality of a scientist's contributions. This unfortunate practice may lower the quality of science, but I do not believe that it is the root cause of fraud. Fourth, it is usually not possible to explain the deeper motivation for the commission of serious scientific fraud within the framework of normal behaviour. I believe that many instances of scientific fraud represent a form of unconscious self-destructive behaviour which may have aggressive components directed against the perpetrator's supervisors, colleagues and institution; indeed, it mocks science as a whole. Fifth, once a single act of outright dishonesty is discovered, the burden should shift to proving that other data presented by the scientist in question were obtained honestly. Sixth, scientific fraud is a crime and must be investigated like any other crime. Not surprisingly, scientists are not trained to be criminal investigators and few are good at it. Seventh, I believe that laboratory directors and mentors are particularly ill-suited to carry out investigations on their own staff, as they have difficulty shedding their roles as teachers of and advocates for their trainees in order to assume the adversarial role demanded of investigators of a possible crime. Eighth, conditions in laboratories can almost always be improved. It is helpful for laboratories to prepare, adopt and adhere to specific written procedures on the conduct of experiments and the reporting of data.

Finally, I do not believe that the environment in which science is carried out is fundamentally flawed, because the overwhelming number of scientists are honest. Society at large is now grappling with the problem of developing a response to terrorism and with the question of how much of our personal freedom we must give up in an effort to minimize it. In certain respects, fraud poses a similarly vexing problem for science. The analogous question in science is how much are we prepared to change the manner in which we live our scientific lives, an integral part of which involves a sense of trust in colleagues, and how much would we lose in making this change in an effort to minimize (it is probably not possible to abolish) scientific fraud? □

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Three-component isotopic heterogeneity near the Oceanographer transform, Mid-Atlantic Ridge

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Tholeiitic basalts collected over an area of less than 100 km² near the Oceanographer transform fault have isotopic compositions interpreted as mixing between three chemically distinct mantle sources. Of the two enriched sources, one has an isotopic composition not previously observed in basalts from the North Atlantic. Its characteristics suggest a model whereby portions of the mantle currently beneath 35° N on the Mid-Atlantic Ridge contain detached fragments of old sub-continental mantle.

OCEANIC ridges and ocean islands, prominent surface features of the Earth's crust, reflect the composition of the underlying mantle from which they are derived. Thus the isotopic compositions of ocean ridge and ocean island basalts are some of the main data used to constrain the distribution and origin of chemical heterogeneity in the upper mantle[1-10]. In general, two classes of models have been used to explain the origin of the observed heterogeneity on ocean ridges. Schilling and coworkers[11-17] have argued for a well-mixed, depleted upper mantle which receives injections of enriched material from discrete plumes rising from the lower mantle. These plumes, or 'hotspots', are recognized at the surface by the presence of ocean islands or very large seamounts. In Schilling's model, which he has called the 'hotspot source - ridge sink' model, the width of the chemical anomaly along the ridge is inversely proportional to the distance of the hotspot from the ridge axis. Localized 'spikes' of enrichment along the ridge are caused by hotspots on the ridge or nearby but off-axis. In contrast to this emphasis on hotspots, others[7,8,10,18-21] favour dispersed heterogeneities of variable shape and size passively embedded in a continually remixed convecting mantle. In this model, enrichment spikes are caused by random sampling of one of the dispersed heterogeneities, with no necessary relationship to hotspots.

The evidence for the former model is based on large-scale sampling of the mid-ocean ridge at 75-100 km spacing in the vicinity of ocean islands. The evidence for the latter model is based on much smaller-scale studies, such as those near the East Pacific Rise, where relatively enriched material can exist without prominent bathymetric anomalies or large hotspots[10,20,22]. Previously, there have been few small-scale studies using multiple radiogenic isotopes (Pb, Sr and Nd) of enriched ridge areas that could either be associated with off-axis hotspots or be sampling dispersed, enriched heterogeneities. Such an area is the Oceanographer Fracture Zone (OFZ). This study investigates the local distribution of basalt types in the OFZ, defines the spatial (and thus temporal) extent of small-scale heterogeneity and considers the heterogeneity in the context of the two competing models. Initial isotopic and trace element results of the study are reported here; complete geochemical data will be presented elsewhere.

The Oceanographer transform fault, located at 35° N on the Mid-Atlantic Ridge, offsets the ridge right-laterally by 130 km (Fig. 1*a*, *b*). The ridge at this latitude lies within the regional geochemical gradient discovered by Schilling[11], which extends from the Azores platform at 39° N ($\mathrm{Ce/Sm_N} > 2.0$) to the Hayes fracture zone near 34° N ($\mathrm{Ce/Sm_N} < 0.8$)[23]. Mixing of material from the Azores plume with depleted upper mantle is the apparent cause of the gradient[11]. To be consistent with this regional gradient, samples from the OFZ area should have flat rare-earth-element patterns, with $\mathrm{Ce/Sm_N}$ of ~1.0. Superimposed on the regional gradient is a 'spike' of geochemical enrichment defined before this work by samples from two dredges, one north of the OFZ and one slightly off-axis to the south of the OFZ, which had $\mathrm{Ce/Sm_N} > 2.0$ and $^{87}\mathrm{Sr}/^{86}\mathrm{Sr} > 0.70320$[13,14]. Rideout and Schilling[24] suggested that this spike was caused by the hotspot that gave rise to the New England seamounts (NES) and that the hotspot signature might also be present in drill holes on 18 and 35-Ma-old crust drilled by Leg 82 of the Deep Sea Drilling Project (DSDP)[25]. One difficulty in interpreting small-scale heterogeneity with the existing data was the ⩾100 km separation between the ridge-axis dredges and the drill holes on older crust.

Results

In this investigation we have undertaken a comprehensive geochemical study of samples from more than 60 stations in the

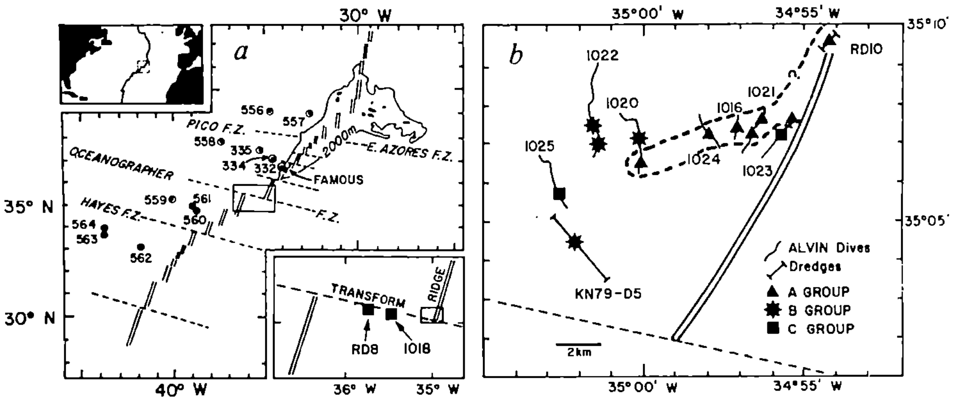

Fig. 1 Sample locations in the OFZ area. *a*, Numbered circles correspond to DSDP drill sites. Holes 332-335 are from Leg 37 and holes 560-564 are from Leg 82. Inset, the location of ALVIN dive 1018 and dredge RD8, both located on the south side of the transform. *b*, ALVIN dive tracks and dredge hauls on scarp northwest of the ridge-transform intersection. The double line represents the ridge axis and the straight broken line represents the transform. The outlined area encircles group A samples only.

Table 1 Major, trace element and isotopic data

Group	Sample		$^{87}Sr/^{86}Sr$	$^{143}Nd/^{144}Nd$	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	Mg no.	K_2O/TiO_2	Ce/Sm_N	Ce/Yb_N	Nd/Sm_N	Ba/Y	Nd/Y
A	1016-1	wr	0.703411±77	0.512882±20	18.747	15.512	38.61	0.64	0.475	2.00	2.87	1.37	6.26	0.519
	1016-1	wr†			18.742	15.508	38.60							
	1020-1-2	wr	0.703479±20	0.512872±25	18.769	15.503	38.55	0.69	0.424	2.07	2.90	1.40	5.74	0.505
	1021-2	wr	0.703512±29	0.512925±15§	18.765	15.491	38.54	0.59	0.520	2.24	3.72	1.47	7.50	0.663
	1021-2	gl	0.703454±51	0.512890±41	18.821	15.495	38.51	0.59	0.551				7.27	
	1021-4	gl	0.703515±31	0.512850±20	18.616	15.493	38.45	0.69	0.423	2.10	3.07	1.36	8.28	0.661
	1023-6-1	wr	0.703399±20	0.512903±26	18.966	15.499	38.71	0.66	0.542	2.29	3.58	1.47	7.56	0.607
	1024-1	wr	0.703460±20‡	0.512860±23	18.820	15.502	38.62	0.70	0.572	2.33	4.24	1.54	9.14	0.713
	1024-1	wr†	(0.703651±27)	0.512896±19§	18.819	15.512	38.68							
	RD10-P4	gl	0.703500±20	0.512871±26	18.583	15.470	38.41	0.61	0.037	2.16	2.95	1.52	5.64	0.599
B	1020-2-1	wr	0.703242±47	0.512995±24	19.210	15.575	38.96	0.59	0.452	1.88	2.83	1.29	5.88	0.520
	1020-2-1	wr†			19.186	15.560	38.92							
	1022-6	wr	0.703188±20‡	0.512990±26	19.538	15.592	39.17	0.68	0.484	2.47	3.53	1.47	8.80	0.560
	1022-6	wr†	(0.703497±31)		19.558	15.626	39.27							
	1022-4-2	wr	0.703073±20	0.513003±46	19.489	15.574	39.08	0.66	0.391	1.90	2.73	1.31	5.58	0.519
	KN79-3-5M	wr			19.452	15.562	40.00	0.62	0.285	1.84	2.61	1.33	4.30	0.536
C	1018-1-2	wr	0.703056±20‡	0.513161±32	18.568	15.518	38.31	0.62	0.267	1.08	1.05	1.05	2.52	0.290
	1018-1-2	wr†		0.513122±12§										
	1023-4	wr	0.703329±60	0.513095±14§	18.639	15.501	38.31	0.71	0.244	1.45	1.59	1.15	2.84	0.328
	1025-1	wr	0.703257±35	0.513038±14	18.989	15.559	38.66	0.69	0.290	1.38	1.99	1.22	2.87	0.447
	1025-1	wr†		0.513005±22§										
	1025-1	gl	0.703093±31	0.512988±24	18.967	15.527	38.57	0.69						
	RD8 P12	wr	0.703210±43	0.513084±16§	18.463	15.552	38.18	0.66	0.293	1.41	1.82	1.14	2.56	0.385
	RD8 P22	wr	0.703187±28	0.513136±15§	18.267	15.537	38.04	0.52	0.255	1.00	1.14	1.02	1.67	0.350

Latitudes, longitudes and depths of samples are given in ref. 27. Samples are designated as follows: wr, whole rocks, gl, glasses, wr†, replicate determination of whole rocks on separate dissolutions. $^{87}Sr/^{86}Sr$ ratios are fractionation corrected to $^{86}Sr/^{88}Sr = 0.11940$ and reported relative to an $^{87}Sr/^{86}Sr$ ratio of 0.70800 for Eimer and Amend $SrCO_3$. Strontium isotope determinations reported with ‡ have been leached in warm 2M HCl to remove seawater Sr. The values reported in parentheses directly under these leaches were determined on unleached whole rocks. All Nd and Pb isotopic compositions were measured on unleached samples. $^{143}Nd/^{144}Nd$ ratios are reported relative to a value of 0.511860 for the UCSD Nd_2O_3 Nd standard and have been fractionation-corrected to $^{146}Nd/^{144}Nd = 0.72190$. $^{143}Nd/^{144}Nd$ ratios measured at the State University of New York, Stony Brook§, are reported in the same manner. In three cases, Stony Brook analyses have been replicated on separate dissolutions to within analytical uncertainty at DTM (Department of Terrestrial Magnetism), showing good agreement between the two laboratories. Pb isotope ratios have been determined on two separate machines at DTM and have been corrected for mass fractionation to NBS 981 on each machine using the values given in ref. 99. One Pb analysis each for 1016-1 and 1020-2-1 (separate dissolutions) was done on each machine and demonstrates good agreement between the two mass spectrometers. The 2σ of the population for multiple determinations of NBS 981 gives a Pb analytical uncertainty of 0.05% AMU^{-1}. In-run precision is at least an order of magnitude better and thus not quoted. Total procedural blanks obtained during the course of this study for 200 mg size samples were 700–1,000 pg for Pb, 90 pg for Nd and 300 pg for Sr. Rare-earth element data were determined at Stony Brook by isotope dilution; for analytical details and precision see Bender *et al.*[29]. Other major and trace element data were obtained by DC plasma spectrometry at Lamont–Doherty. Mg nos have been calculated assuming Fe as $0.85\ Fe^{2+}$.

OFZ area. A more thorough investigation of heterogeneity has been made possible here because of the ALVIN submersible study of Fox *et al.*[26] and dredges from other cruises. All the samples were collected north of the transform within 10 km of the ridge (Fig. 1*b*)[27] with the exception of three samples collected on the south side of the transform ~50–60 km west of the ridge axis (Fig. 1*a*). The samples are mostly phyric whole-rocks showing little alteration in thin section. Glassy samples are rare but glass was analysed when present. Table 1 shows that some of the unleached whole-rock samples have a higher $^{87}Sr/^{86}Sr$ than leached whole-rocks or glasses. Because of the scarcity of glasses, we draw our petrological interpretations largely from the Pb and Nd isotope data although they are supported by the Sr isotope data.

The isotopic data and some major element and trace element ratios are summarized in Table 1 and Figs 2*a–e* and 3*a*, *b*. All samples are tholeiitic basalts. Based on trace element ratios, none of the samples are 'normal' depleted ocean-ridge basalts, that is, none have $Ce/Sm_N < 1$ and $Ba/Y < 1$ (see Fig. 2). The most depleted samples from the region have slightly enriched chemistry with Ce/Sm_N of 1.0–1.5 and Ba/Y of 1.6–3.0, in accord with the regional gradient extending south from the Azores[11]. The enrichment spike inferred from two previous dredge hauls[14] is strikingly confirmed by the new data, with two-thirds of the samples analysed having chemistry enriched over the regional gradient and about half of the samples with Ce/Sm_N as high as the most enriched basalts ever recovered from the global system of ocean ridges.

The trace element data might be interpreted as evidence for a single enriched source feeding the depleted mantle beneath the ridge in the OFZ area. The isotopic data, however, show that the trace element enrichment is due to two distinct enriched sources. The two enriched groups of samples have been designated groups A and B with the most depleted group as group C. The samples in groups B and C exhibit a continuous variation in isotopic composition, which falls within the field previously defined for this region of the North Atlantic by both on- and off-axis samples (Fig. 3). We have arbitrarily divided this C–B continuum into two groups to allow comparison of the most enriched samples from it, group B, to the more homogeneous group of enriched samples, group A. Groups A and B have nearly indistinguishable concave upwards rare-earth-element patterns, in contrast to the flat rare-earth-element patterns of group C (Fig. 2*a–c*). In Table 1 and Figs 2*d*, *e* and 3, the group A samples are isotopically distinguished by (1) lower $^{143}Nd/^{144}Nd$ and $^{206}Pb/^{204}Pb$ but higher $^{87}Sr/^{86}Sr$ at similar enrichments in Ce/Sm, (2) lower $^{143}Nd/^{144}Nd$ for the same $^{206}Pb/^{204}Pb$, and (3) slightly higher $^{208}Pb/^{204}Pb$ and lower $^{207}Pb/^{204}Pb$ for the same $^{206}Pb/^{204}Pb$.

Discussion

The isotope data require melting of a source containing distinct components that have been, at some point in their history, chemically isolated for a minimum of 600 Myr. Given that the spreading age of the exposed crust in the OFZ region is only 1–5 Myr, the isotopic variability must reflect ancient mantle events. In terms of isotopic components, three distinct compositions are needed to explain the isotopic variability displayed by the OFZ data. One component has the characteristics of normal, depleted MORB (mid-ocean-ridge basalt) which is, however, not tapped as a pure end-member in this region. The second component is a high-Pb, low-Nd component similar to the isotopic composition represented by the Azores and the New

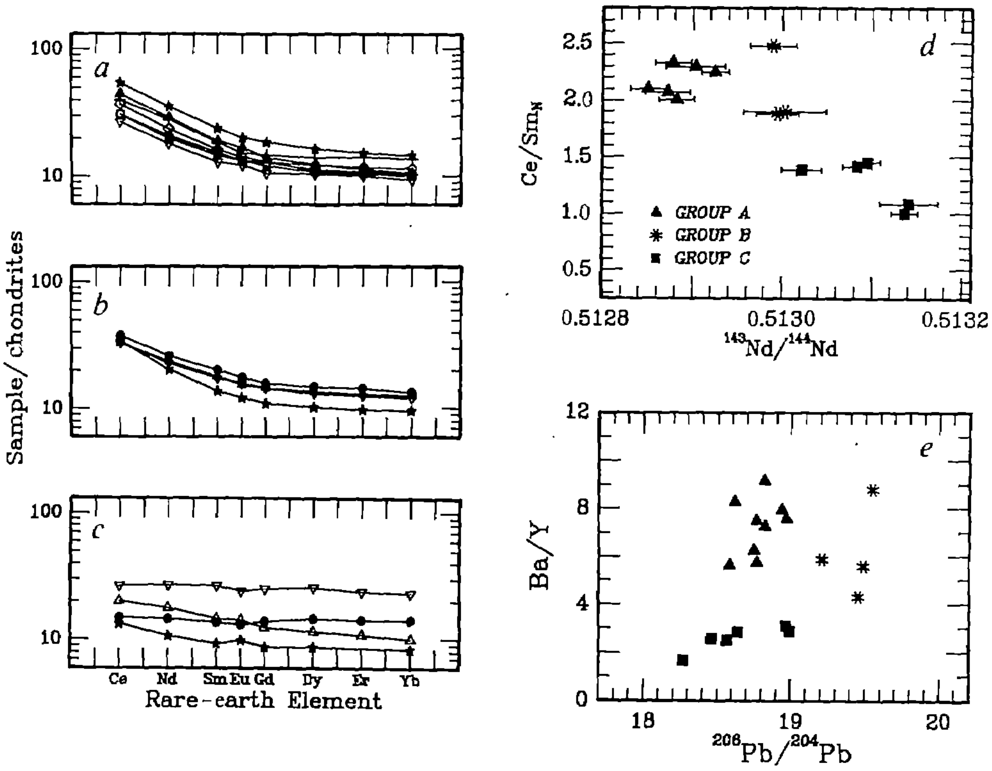

Fig. 2 *a–c*, Isotope dilution, rare-earth-element patterns for the three groups (A–C respectively) of tholeiites in the OFZ area. *d*, *e*, Trace element ratio plotted against isotope ratio for OFZ tholeiites (▲, group A; ✱, group B; ■, group C). The analytical uncertainty for $^{143}Nd/^{144}Nd$ is given by error bars; the analytical uncertainty for $^{206}Pb/^{204}Pb$ is 0.05% AMU^{-1} or about 1/2 the width of the data-points.

England seamounts (NES). The third component is a low-Pb, low-Nd component necessary to explain the group A samples (Fig. 3). This component has not been observed in the North Atlantic and, as far as is currently known, is not represented by any of the North Atlantic hotspots.

Inspection of Fig. 1*b* shows that the geographic distribution of the three chemical groups is not random. The enriched group A samples form a band which is not quite perpendicular to the ridge axis and extends over a length of about 12 km. The group B samples occur exclusively in a band approximately parallel to the ridge, about 10 km off-axis; and the group C samples occur at the westernmost and easternmost extremes of the study area. This distribution suggests that the mantle sources may not be distributed only as fine-scale (cm to m) veins but that there are discrete domains with volumes of the order of at least tens of km^3.

The lack of spatial systematics with respect to the transform suggests that the chemical diversity is not primarily transform-related. There is no direct relation between proximity to the transform and the extent of enrichment, as might be expected from the 'transform fault effect'[28,29]. Indeed, the two samples from stations on older crust which are closest to, but just south of, the transform are the group C samples, the least enriched chemical type. The distribution of chemical types does not appear to be affected by the transform either. The diversity in the small area north of the transform is greater than most chemical discontinuities documented across some transforms.

The chemical heterogeneity in the OFZ area is unusual in the absolute magnitude of the isotopic diversity, the type of components sampled and the presence of three chemically distinct components in a small area. In the FAMOUS region at 36° N on the Mid-Atlantic Ridge, the isotopic compositions of the basalts were found to be homogeneous[30,31]. In other areas more diverse than FAMOUS, such as across the Kane fracture zone near 24° N on the Mid-Atlantic Ridge[32] and just north of the Siqueiros fracture zone on the East Pacific Rise[33], the magnitude of isotopic variability is less and the length scale over which it is developed is greater than in the OFZ area. The one small piece of ocean crust that exhibits a similar range of variability is seamount 6 near the East Pacific Rise[10,20]. Compared with data from this seamount, the OFZ data show a similar range in Nd isotopic composition and a slightly smaller range in light rare-earth-element enrichment. Both seamount 6 and the OFZ area lie on the upper bounds of the oceanic mantle correlation between total isotopic variability and length scale[34], so neither is unique in a global sense. The OFZ area, however, does have the first-documented existence of three different chemical components in a small area. Moreover, this three-component mantle heterogeneity observed in the OFZ area does not appear to involve exactly the same end-members as the three components[35,36] or multiple components[7,37] proposed for other MORB and ocean island data sets. This is because of the unradiogenic $^{143}Nd/^{144}Nd$, $^{206}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ of the group A samples, coupled with their high $^{87}Sr/^{86}Sr$.

North Atlantic mantle heterogeneity. The group C to B isotopic variation in the OFZ area is similar to the regional trends from a larger area of the crust generated at the ridge over the last 35 Myr and by the two nearest hotspots active since the last 60–80 Myr (for instance Azores and NES). The unique isotopic composition of the group A samples does not fit the regional pattern and will be considered separately below. Most North Atlantic MORBs exhibit a systematic, correlated variation in Pb, Sr and Nd isotopic composition[37–42]. The group B and C tholeiites also show this correlation, being encompassed by the MORB field including DSDP Legs 37[43,44] and 82[24,45,46] and the FAMOUS area on Pb–Nd, Pb–Pb isotope diagrams (Fig. 3*a*, *b*). Chemically, we refer to this trend as the North Atlantic MORB mantle. The isotopic composition of North Atlantic oceanic rocks is extended along the North Atlantic MORB mantle trend to more extreme values by the two hotspots in the area (Fig. 3*a*, *b*).

In relation to the hotspot source - ridge sink model for this region of the North Atlantic[11,15,24,44], the lack of normal depleted MORBs in the OFZ area has been explained by the down-ridge geochemical gradient away from the Azores plume[14,15], whereas the presence of an anomalous enrichment spike above the gradient has been attributed to the NES hotspot[15,24]. The new data on the group B samples are in accord with this model because their sources are enriched in components isotopically similar to the off-axis NES hotspot. However, the isotopic composition of group B is not a particularly good constraint on the importance of the NES hotspot, because most samples from the

North Atlantic fall in the C-B trend, and the group B enrichment could be caused by a component from the Azores, the NES hotspot or some unknown source similar to that which caused the enrichment in hole 558 of Leg 82[24,45,46]. Group B cannot be uniquely connected to the NES hotspot and the role of the NES hotspot in the OFZ enrichment can only be inferred from reconstructions of past positions of plates and hotspots[24,47].

Thus for the group C-B trend within the OFZ area, there may be three geographically distinct components, but there are only two chemically distinct components. The role of the Azores as a hotspot source for the OFZ area is implied by the coincidence in Ce/Sm and ^{87}Sr/^{86}Sr of the group C samples with the down-ridge geochemical gradient[11]. The role of the NES hotspot in group B enrichment is based on proximity, isotopic composition and the idea that it would not be plausible for the hotspot producing a smooth down-ridge gradient to produce a sharp down-ridge spike. Both of these enriched components are mixed with depleted MORB; hence three geographically distinct components are present. However, only two chemically distinct reservoirs can be resolved with the isotope data because the two separate hotspots (Azores and possibly NES) and the group B samples fall along the same isotropic trends, which extend towards a high ^{206}Pb/^{204}Pb and ^{208}Pb/^{204}Pb, low ^{143}Nd/^{144}Nd component.

The isotopic composition of the group A samples cannot be explained by simple mixing of the mantle source for groups C and B with any of the enriched mantle sources present in the North Atlantic. The group A samples are distinct from the groups B and C mixing curves (Fig. 4*a*–*c*) and are characterized by Pb and Nd isotopic compositions that lie significantly off the MORB-Leg 82-Azores-NES isotope trend (Fig. 3*a*). Even mixing of MORB with a high ^{206}Pb/^{204}Pb, low ^{143}Nd/^{144}Nd component such as Sao Miguel, cannot explain the group A samples. The seven group A samples show negative correlations in Pb-Sr isotopes, and positive correlations for Pb-Nd isotopes. Compared with many enriched MORBs, they have Pb isotopic compositions that are low in ^{206}Pb/^{204}Pb, ^{207}Pb/^{204}Pb and ^{208}Pb/^{204}Pb. These isotopic characteristics do not correspond with any currently observed North Atlantic hotspots. Without an appropriate off-ridge hotspot with group A chemical characteristics, the hotspot source–ridge sink hypothesis seems a tenuous explanation for the observed enrichment. However, this model cannot be completely ruled out without more comprehensive isotopic analysis of other possible off-axis hotspots in the region.

An alternative hypothesis is that enrichment of the group A source may be due to passive enriched domains embedded in a matrix of North Atlantic MORB mantle. This random domain model for the 35° N area was dismissed by Rideout and Schilling[24] in favor of the hotspot source–ridge sink idea because all isotopic enrichment in the OFZ area was previously believed to be similar to the off-axis NES hotspot. One way to examine further the feasibility of the passive heterogeneity model is to attempt to identify the parent reservoir of the dispersed domain.

Enrichment of the oceanic mantle by incorporation of pelagic sediments[6,39] during subduction can be ruled out because it would yield far too high ^{207}Pb/^{204}Pb to account for the isotopic composition of group A (Fig. 5). Incorporation of upper continental crustal sediments[48] can be eliminated using similar reasoning. Few of the ocean ridge or island samples so far analysed can specifically account for the enrichment seen in the group A samples (Fig. 5). Portions of the Pacific or Indian MORB reservoirs appear to have low enough ^{206}Pb/^{204}Pb and ^{207}Pb/^{204}Pb but these samples have too high ^{143}Nd/^{144}Nd[34,38,40–42,49–52]. Many ocean island reservoirs have low enough ^{143}Nd/^{144}Nd but with the exception of Iceland (not shown) and Hawaii, all of them are like Tristan and Gough, for example, in having too high ^{207}Pb/^{204}Pb (Fig. 5). An Iceland-like reservoir is unlikely because the group A samples have higher ^{87}Sr/^{86}Sr and lower ^{143}Nd/^{144}Nd than any Icelandic rocks yet analysed[34], whereas a Hawaii-like reservoir (except for th Koolau volcanic series) can be discounted because of its inappropriately high ^{143}Nd/^{144}Nd for its ^{206}Pb/^{204}Pb. The Koola series on Hawaii[53] does have an isotopic composition appropriate to explain the chemistry of the group A samples. However the Hawaiian hotspot is the largest isolated hotspot on Eart and its chemistry is different from the currently known Nort Atlantic hotspots. Because there is no Hawaii-like hotspot i the North Atlantic with which to link the group A samples, i may be worthwhile to consider a randomly disperse heterogeneity model to explain the incorporation of this unusua enriched component.

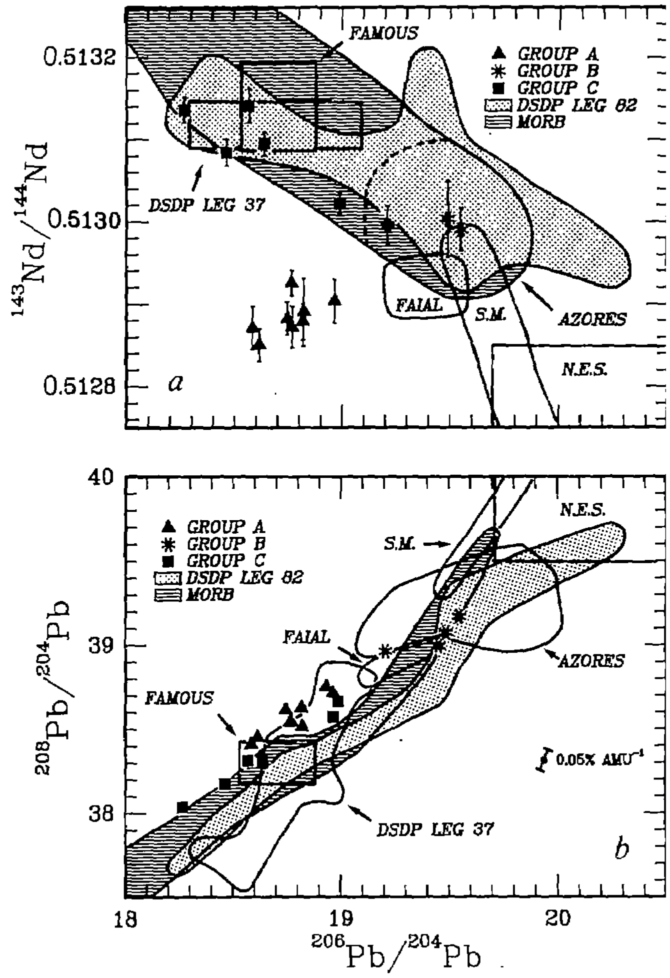

Fig. 3 Nd–Pb (*a*) and Pb–Pb (*b*) isotope compositions for Oceanographer tholeiites compared with other basalts from the nearby North Atlantic. Data sources as follows: MORB field exclusive of Indian Ocean[38,34,39–42,61,88], Azores Islands exclusive of Faial and Sao Miguel[34,89], Faial[6,89], Sao Miguel (S.M.)[89,90], DSDP Leg 82[24,45,46], DSDP Leg 37[43,88], FAMOUS[30,31] and New England seamounts (N.E.S.)[34,91]. Analytical uncertainty for ^{143}Nd/^{144}Nd is shown in *a*; analytical error for Pb isotopes is shown in *b*.

A sub-continental mantle source for OFZ heterogeneity? Th sub-continental lithospheric mantle is an enriched reservoir wit the chemical characteristics necessary to explain the enrichmen of the group A tholeiites. The composition of the subcontinenta mantle is thought to be generally depleted in basaltic components[54–56] although it may become subsequently enriched b metasomatism involving melts[57]. Typically, it has a highly variable trace element[58,59] and isotopic composition[60–62]. Figur 5*a*–*c* shows the isotopic composition of the more unradiogeni portions of the Archaean to Proterozoic subcontinental mantl thought to make up the lower part of the Wyoming[63–65] an Kaapvaal cratons[62]. It should be noted that more radiogeni portions of the subcontinental mantle from these cratons hav

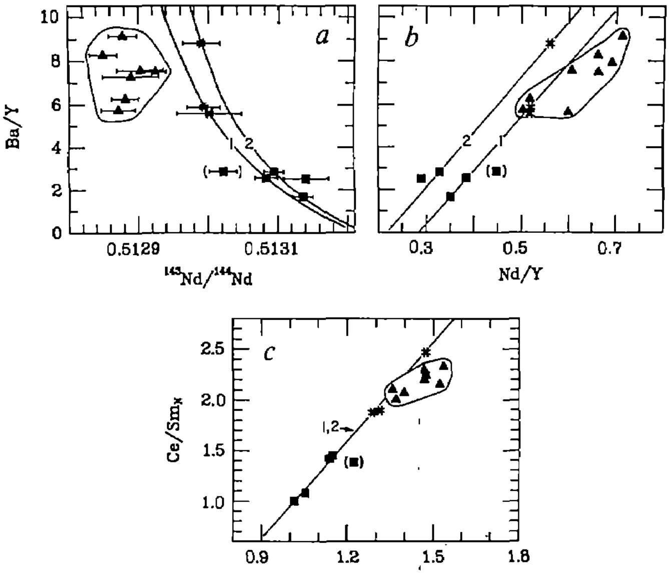

Fig. 4 Trace element ratio-isotope ratio mixing calculations; symbols as in Fig. 3. **a**, The curves through the group B and C tholeiites are calculated with the mixing equations of Langmuir[92] using analyses for samples of similar Mg no. This method avoids fractionation effects on concentration and carries no prior assumptions about source concentration or composition. Curve 1 is calculated from samples 1022-4-2 (13.5 p.p.m. Nd, 26.0 p.p.m. Y) and P12 (10.4 p.p.m. Nd, 27.0 p.p.m. Y) and curve 2 is calculated from samples 1022-6 (12.1 p.p.m. Nd, 21.6 p.p.m. Y) and 1023-4 (6.36 p.pm. Nd, 19.4 p.p.m. Y). Sample in parenthesis is 1025-1, which does not fall on either mixing curve. *b*, Companion plot supporting mixing of group B and group C mantle sources. Curves 1 and 2 are drawn through the samples used to plot the mixing curves. Note that multiple mixing curves are required by the Ba/Y data. In addition, the group A samples define a different slope, emphasizing the involvement of a third mantle reservoir. *c*, Companion plot using only rare-earth-element data (accompanies Fig. 2*d*).

been omitted from Fig. 5. This was done to clarify the figure and also because of the inability of the more radiogenic subcontinental mantle to explain the group A tholeiites. Mixing a relatively small amount (8–12%) of subcontinental mantle with a North Atlantic MORB mantle along the group C to group B trend will lower the ^{143}Nd/^{144}Nd, ^{206}Pb/^{204}Pb, ^{207}Pb/^{204}Pb and ^{208}Pb/^{204}Pb while raising the ^{87}Sr/^{86}Sr (Fig. 5*a–c*) enough to account for the composition of the group A samples. The large variability in composition of the sub-continental mantle means that there is no unique mixing curve or end-member composition. However, the family of possible mixing curves using sub-continental mantle as an end-member dictates that the North Atlantic MORB mantle end-member must be considerably enriched in NES-like components because the sub-continental lithosphere is low in both ^{206}Pb/^{204}Pb and ^{207}Pb/^{204}Pb (Fig. 5*a*, *b*). These features are consistent with a model for the group A mantle source as an enriched domain carrying sub-continental lithospheric mantle embedded in a matrix of North Atlantic MORB mantle. The sub-continental mantle has previously been implicated in explaining MORB heterogeneity[51,66], and the isotopic and trace element composition of the group A tholeiites perhaps adds to the evidence that removal and recycling of pieces of ancient sub-continental mantle into the convecting mantle could be responsible for some small-scale heterogeneities of the MORB mantle.

The distribution of heterogeneities as dispersed enriched domains is consistent with ideas about how parts of the subcontinental lithosphere might be incorporated into the convecting mantle. Based on seismic studies[54], heat flow and thermal modelling[67,68], geodynamic constraints[69], flood basalt chemistry[60,70,71], xenolith thermobarometry[72], mantle xenolith chemistry[60] and diamond inclusions in kimberlite[73], a mantle keel makes up approximately two-thirds or more of the present cratonic lithosphere. This 100–200 km-thick mantle root was frozen out of mantle convection beneath the cratonic crust during or shortly after continent stabilization and has remained physically isolated from the convecting mantle for 10^9 yr time periods. The continental lithosphere must be affected by rifting, subduction around its margins and mantle upwelling at hotspots. These processes have the potential to lead to destabilization of previously cool lithospheric mantle. Some portions of the lithospheric mantle could be removed and entrained in the flowing mantle. The subcontinental mantle, delaminated in this manner, has been suggested to cause crustal melting[74], to provide dispersed heterogeneities in the convecting mantle[75], to give rise to ocean island basalts[61,76,77] and to contribute to certain ocean island mantle sources[78–80].

An estimate of the size of the enriched mantle domain in the OFZ area is given by the distribution of the group A samples (Fig. 1*b*). However, its true shape and original size when detached from the sub-continental lithosphere are not constrained. A heterogeneity should become thinner with age[81,82], but how heterogeneities survive in a convecting mantle and whether they could survive since the Archaean or Proterozoic is highly dependent on how convection is modelled. Recent calculations for whole mantle convection[83] show 1–2 Gyr survival times. This result differs from other studies that show the upper mantle will be somewhat mixed within 0.5–1 Gyr[75,82]. If old heterogeneities can survive for long periods, then apparently the OFZ area shows an example of one such heterogeneity. If mantle convection is more effective in stirring the mantle towards homogeneity, then a mechanism for recycling old pieces of sub-continental mantle, such as delamination, provides an explanation of how the isotopic age of a heterogeneity could exceed the limit on its apparent physical age.

The implications of enriched domains of sub-continental lithosphere for explaining heterogeneity in the oceanic mantle are profound. Because only Precambrian materials can preserve significant primary variations in ^{235}U/^{204}Pb, this mechanism could contribute to the long-recognized antiquity of the variation in ^{206}Pb/^{204}Pb against ^{207}Pb/^{204}Pb of the oceanic mantle[3,5,84,85]. It could also be responsible for specific areas anomalous in ^{207}Pb/^{204}Pb[9,36]. If such dispersed, enriched heterogeneities exist in the MORB mantle, are plumes always necessary to explain enrichment spikes on the ridge? For example, such a model could provide an alternative to the hotspot source - ridge sink model for the South Atlantic[15]. Hawkesworth *et al.*[78] have suggested that the sub-continental mantle at the margin of the South Atlantic was responsible for the unusual Pb isotopic chemistry of the South Atlantic hotspots (Discovery, Tristan, Gough)[5] and this signature has been shown to be faithfully reproduced in the MORB Pb isotope data[17]. During the opening of the present South Atlantic any detached pieces of sub-continental mantle could have been dispersed along convective flow lines. At present, enriched domains with similar isotopic composition should be sampled at the ridge as well as ocean islands, as long as they are the same spreading flow line.

Conclusions

Tholeiites from the OFZ area display much heterogeneity in trace elements and Sr, Nd and Pb isotopes: they are resolvable into three different chemical components. Tholeiites from groups C and B form a continuous enrichment trend, the group C end-member being consistent with the regional enrichment gradient from the Azores southward along the Mid-Atlantic Ridge. The group B samples of the trend are enriched in trace elements and isotopes relative to the down-ridge gradient. Several of the hotspots in this vicinity of the North Atlantic have the isotopic composition to cause this enrichment, but the New England seamount hotspot seems to be the most logical,

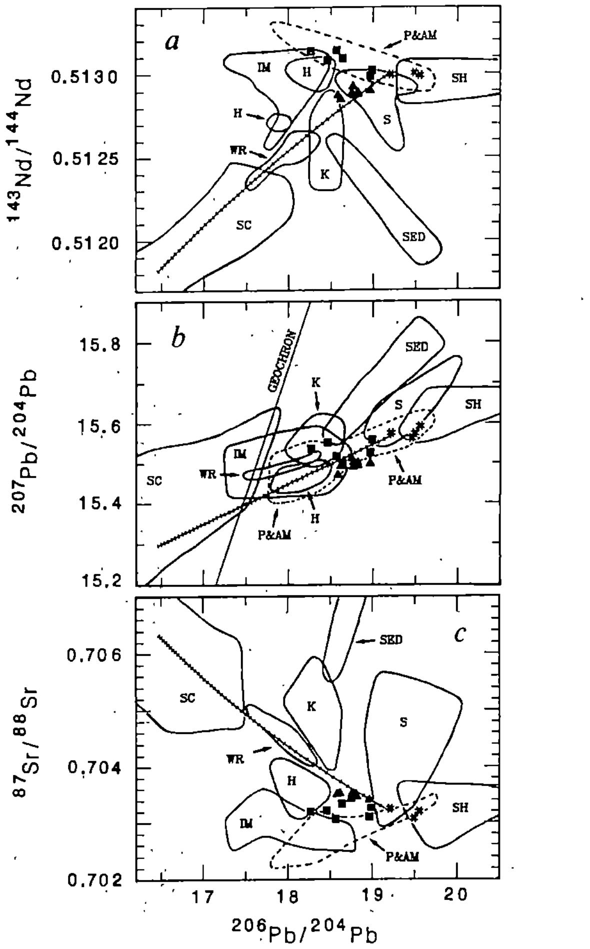

Fig. 5 Comparison of Nd-Pb (*a*), Pb-Pb (*b*) and Sr-Pb (*c*) data for OFZ area tholeiites (▲, group A; ✻, group B; ■, group C) with the isotopic composition of proposed mantle reservoirs and sediments. Oceanic mantle reservoirs are grouped generally in accord with ref. 37, but data are taken from the literature: Saint Helena, Ascension, Australs (SH)[5,6,37,93]; Societies, Marquesas, Sao Miguel (S)[6,88,89,93,94]; pelagic and continental sediment (SED)[95,96]; Kerguelen, Tristan, Gough (K)[5,6,97]; Walvis Ridge (WR)[66]; Hawaii (H)[53,98]; Atlantic and Pacific MORB (P&AM, refs in Fig. 3); Indian MORB (IM)[42,49-52]; and sub-continental mantle (SC). The sub-continental mantle field includes relatively unradiogenic lamproites[64]; kimberlites[62], alkaline and subalkaline volcanic rocks[63], high-alumina tholeiites and high-magnesian andesites[65]. These samples represent the estimated isotopic composition of sub-crustal lithospheric portions of the Wyoming and Kaapval Cratons. Note that the composition of the sub-continental mantle displays a huge isotopic variability particularly in $^{207}Pb/^{204}Pb$ and it contains significant portions that have radiogenic $^{87}Sr/^{86}Sr$ but highly unradiogenic $^{143}Nd/^{144}Nd$, $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$. The Koolau volcanic series is the smaller of the two Hawaiian fields in *a* and makes up the less radiogenic $^{206}Pb/^{204}Pb$ end of the Hawaii field in *b* and *c*. The solid line with ticks at 2% increments displays mixing between a MORB mantle somewhat enriched in B components (30 p.p.m. Sr, 3 p.p.m. Nd, 0.4 p.p.m. Pb, $^{87}Sr/^{86}Sr = 0.70320$, $^{143}Nd/^{144}Nd = 0.51301$, $^{206}Pb/^{204}Pb = 19.20$, $^{207}Pb/^{204}Pb = 15.57$, $^{208}Pb/^{204}Pb = 38.75$) and estimates of sub-continental mantle (45 p.p.m. Sr, 4 p.p.m. Nd, 0.8 p.p.m. Pb, $^{87}Sr/^{86}Sr = 0.70629$, $^{143}Nd/^{144}Nd = 0.51184$, $^{206}Pb/^{204}Pb = 16.50$, $^{207}Pb/^{204}Pb = 15.30$, $^{208}Pb/^{204}Pb = 37.08$).

based on past plate and hotspot positions[24,47]. A hotspot source-ridge sink model[15] can account for the presence of this enriched component on the Mid-Atlantic Ridge in the vicinity of the OFZ.

The second enriched component in the OFZ area, seen in the group A tholeiites, has not previously been observed in North Atlantic MORBs. Compared to MORBs, it is characterized by relatively unradiogenic $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$ and $^{143}Nd/^{144}Nd$ but radiogenic $^{87}Sr/^{86}Sr$. The spatial restriction of tholeiites tapping this source to crust between 0 and 10^6 yr shows that this heterogeneity exists in the mantle on a scale larger than the scale of melting[86,87]. Its unique isotopic composition does not correlate with the known compositions of any known North Atlantic hotspots and may reflect an isolated anomalous region entrained in the upwelling MORB mantle. The unusual chemistry of the group A samples at 35° N can be modelled by mixing 8-12% of Archaean to Proterozoic sub-continental lithospheric mantle into North Atlantic MORB mantle which is already enriched with NES-like components. Sporadic incorporation of enriched domains of sub-continental mantle may be an important mechanism to explain some small-scale or even some large-scale mantle heterogeneities that are inferred from the geochemical study of the oceanic crust.

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Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic β cells

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Transgenic mice expressing simian virus 40 T antigen under control of the insulin gene regulatory region vary in their response to this protein. Each lineage is characteristically either tolerant to T antigen, or not, in which case autoantibodies arise with high frequency, and lymphocytes infiltrate and disrupt the pancreatic islets. Both non-tolerance and the autoimmune response appear to result from delayed onset of T antigen expression during β cell development.

THE way in which the immune system discriminates between self and non-self and maintains a state of tolerance to self-antigens is not fully understood. It is thought that during lymphocyte differentiation and maturation the self-reactive cells of both the cellular and humoral immune system are either physically deleted, functionally inactivated, or have their activity continuously suppressed. The complexity of the immune system, and of the experiments that have sought to describe it, has resulted in a number of theories on the mechanisms of self-tolerance[1–3]. The failure of self-tolerance and production of high levels of autoantibodies are characteristic of a variety of chronic disease states[4]. A broad spectrum of antigenic targets are recognized, including hormones, DNA, ribonucleoprotein complexes and nuclear proteins. Autoantibody production may also be important in the pathogenesis of insulin-dependent (type I) diabetes mellitus (IDDM). In IDDM, 60–85% of newly diagnosed patients have islet-cell specific autoantibodies[5]. The appearance of islet-cell autoantibodies implies that β cells are being destroyed; this may occur up to eight years before the clinical manifestation of the disease[6].

Recently one of us (D.H.) established several lineages of transgenic mice carrying integrated copies of a hybrid gene comprising the upstream regulatory sequences of the rat insulin II gene linked to protein coding information for the simian virus 40 (SV40) large T antigen[7]. Immunohistological analysis of pancreatic tissue from transgenic mice showed that large T antigen was expressed in a tissue-specific manner, being found exclusively in the β cells of the islets of Langerhans. Immunochemical and immunohistological studies on a variety of other tissues have not revealed additional sites of T antigen synthesis. The expression of large T results in hyperplasia of the pancreatic islets and formation of β cell tumours. During these studies we became interested in the immunological consequences of transferring foreign genes into the mouse germ line. One might expect the immunological response to a protein produced by a stably integrated, inherited transgene to be similar to that of other self-proteins, that is, tolerance, but, surprisingly, this is not always the case. In three of the four mouse lineages transgenic for the insulin-promoted SV40 T antigen gene, a high incidence of serum autoantibodies to large T antigen is observed.

Autoantibodies to SV40 large T

Sera were isolated from randomly selected mice of various ages (six weeks to ten months) comprising control (B6D2F1/J strain) animals and representative animals of the RIR-Tag, RIP-Tag2, -Tag3 and -Tag4 transgenic lineages[7]. The presence of serum autoantibodies to large T antigen was assayed by immunoprecipitation of T antigen from extracts of biosynthetically-labelled COS cells, a transformed primate cell line constitutively expressing T antigen[8]. Immunoprecipitates were

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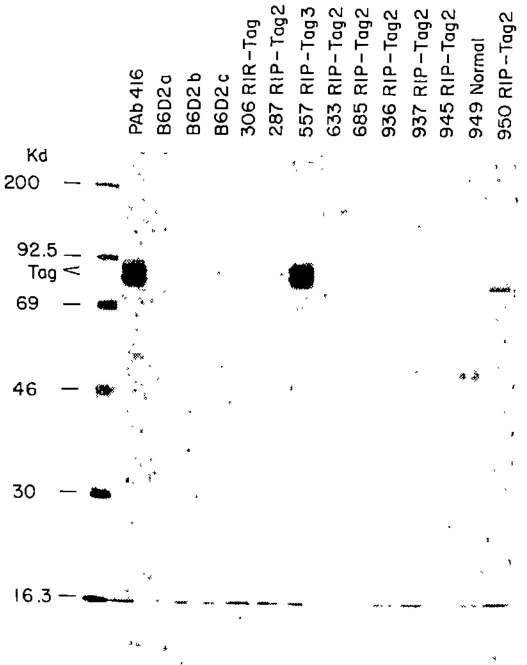

Fig. 1 Autoantibodies of SV40 large T antigen in sera from transgenic mice. Detergent-solubilized lysates of ^{35}S-methionine-labelled COS cells were immunoprecipitated with a large-T antigen-specific monoclonal antibody (PAb416)[9], sera from B6D2 mice (tracks a–c) or with sera obtained from randomly selected mice of the RIR-Tag, RIP-Tag2 and -Tag3 transgenic lineages. Serum from an animal (949) that was negative for integrated copies of the insulin promoter-SV40 T antigen gene (a result of a mating between a normal and a transgenic mouse) was included. Immunoprecipitations were analysed by electrophoresis on 10% SDS-polyacrylamide gels and gel autoradiography.

Methods. Immunoprecipitations were performed as described[20], with minor modifications. Semi-confluent monolayers of COS cells[8] were labelled 4 h with ^{35}S-methionine (Amersham) in Dulbecco's modified Eagle medium (minus methionine) and cell extracts prepared by lysis with 1% NP-40. Lysates were precleared overnight by incubation at 4 °C with 10 μl of normal rabbit serum and 100 μl of a 10% suspension of fixed *Staphylococcus aureus* Cowan I strain cells. Aliquots of lysate supernatant were reacted with 1 μl of mouse serum for 30 min at 4 °C. Rabbit anti-mouse immunoglobulin antisera (1 μl) was added and the incubation continued for 30 min. Immune complexes were collected by absorption to protein A-Sepharose beads (Pharmacia), washed, eluted and analysed on 10% SDS-polyacrylamide gels[20].

analysed by SDS-polyacrylamide gel electrophoresis and gel autoradiography (Fig. 1). A characteristic large-T-antigen doublet of relative molecular mass 85,000–90,000 (M_r 85–90K) is immunoprecipitated by the T antigen-specific monoclonal antibody PAb416 (ref. 9). Sera from control mice and from the RIP-Tag2 lineage did not immunoprecipitate detectable amounts of T antigen. In contrast, strongly immunoprecipitating antibodies are found in serum from 557, a RIP-Tag3 mouse. Similarly, weakly immunoprecipitating antibodies are observed in 306, a RIR-Tag animal, reflecting a low antibody titre.

The pattern of autoantibody incidence is clearer when the results from the random screen of transgenic mice are tabulated (Table 1). With one exception (a weak positive), sera obtained from RIP-Tag2 mice did not immunoprecipitate T antigen, but sera from RIP-Tag3, RIP-Tag4 and RIR-Tag mice did. The high incidence (61%) of autoantibody-positive sera from RIP-Tag3 animals compared to these from RIP-Tag4 animals (35%) is intriguing, as the relative frequencies were stable in successive generations, and are apparently characteristic of the lineages.

Table 1 Frequency of autoantibodies to SV40 large T antigen in sera from different transgenic mouse lineages

Lineage	No. of mice sampled	No. of mice autoantibody positive
RIP-Tag2	26	1* (4%)
RIP-Tag3	71	43 (61%)
RIP-Tag4	34	12 (35%)
RIR-Tag	11	3 (27%)

Serum was isolated from randomly selected transgenic mice of various ages and screened by immunoprecipitation for the presence of antibodies against SV40 large T antigen, as described (Fig. 1). Twenty of the RIP-Tag3 and -Tag4 mice were H-2 typed, in an assay of complement-mediated lysis of splenocytes using H-2 type specific monoclonal antibodies (a gift of U. Hammerling). Thirteen were H-2^{b}/H-2^{b}, six were H-2^{b}/H-2^{d}, and one was H-2^{d}/H-2^{d}. There was no correlation between the H-2 haplotype and the presence of autoantibodies. This is consistent with the observed ability of both haplotypes to respond to congenic SV40 transformed cells[21–23], and to purified large T-antigen[21].

* Weak positive reaction.

The appearance of T antigen autoantibodies is age related; 48% (12/25) of RIP-Tag3 mice under six months of age had detectable autoantibody responses, whereas 67% (31/46) of the mice over six months of age had serum autoantibodies against T antigen (data not shown). Autoantibodies specific for T angiten have never been observed in sera taken from normal (non-transgenic) mice.

Autoimmune diseases are often associated with specific haplotypes of the major histocompatibility complex, although in general strict causal relationships have not been established. The original RIP-Tag transgenics were F$_2$ hybrids between the inbred strains C57Bl/6J (H-2^{b}) and DBA/2J (H-2^{d}). These founder mice were backcrossed to C57Bl/6J to establish lineages. It is therefore possible that differences in the H-2 haplotypes of the different lineages are affecting the autoimmune response. To address this, mice from each lineage were analysed for both autoantibodies and H-2 type (Table 1). No correlation between the two was observed, nor has continuous breeding altered the frequency of autoantibodies. These data argue against a strict linkage to either H-2 haplotype.

No link between autoantibodies and tumours

These lines of transgenic mice heritably develop highly vascularized tumours that are composed of β cells expressing large T antigen, raising the possibility that the appearance of autoantibodies to T antigen is a direct consequence of tumour formation. To assess this possibility, 27 mice of the RIP-Tag3 and -Tag4 lineages were killed, and their pancreases examined under a dissecting microscope for the presence of vascularized tumours. Serum was isolated and analysed for T-antigen specific antibodies. The results for six mice are shown in Fig. 2. Autoantibodies were not observed in sera from three out of five tumour-bearing mice, whereas two mice showed both autoantibodies and tumours. The sixth mouse shown in Fig. 2, 1806, displayed a strong antibody response to large T in the absence of identifiable tumours. This has proved to be a common observation as these analyses have been extended to mice in the RIR-Tag and RIP-Tag4 lineages, as well as to other RIP-Tag3 mice. In the study of 27 RIP-Tag3 and -Tag 4 mice, 17 were autoantibody positive, of which nine had tumours and eight did not. Similarly, four of the ten autoantibody-negative mice had tumours. Of the mice under six months old, four out of seven were autoantibody positive, and none of the seven had tumours. Thus it seems that the humoral autoimmune response can precede tumour formation, and that the induction of autoantibodies against T antigen is not an obligatory consequence of the presence of β cell tumours.

Self-tolerance assays

A number of phenotypic differences between these transgenic lineages have been observed. Mice of the RIP-Tag2 lineage develop multiple β-cell tumours and die between ten and sixteen weeks of age. In contrast, mice of the RIR-Tag, RIP-Tag3 and -Tag4 lines develop tumours more slowly, and live for six to twelve months. As mentioned previously, there appears to be an increased incidence of T-antigen-specific autoantibodies in sera taken from older transgenic mice. The absence of autoantibodies in the sera of RIP-Tag2 mice might therefore simply reflect the premature death associated with this lineage. To examine this question, four normal mice and four transgenic mice from each lineage, all six to eight weeks of age, were immunized with immunoaffinity-purified large T antigen, and their primary and secondary antibody responses were analysed. The control mice, together with all individuals in the RIR-Tag, RIP-Tag3 and -Tag4 lineages, all mounted a readily detectable primary antibody response as a result of a single immunization with T antigen (Fig. 3). In contrast, only one individual (RIP-Tag2c) of the four RIP-Tag2 mice immunized displayed a detectable, weak, primary response.

The secondary immune responses of the different lineages to a subsequent immunization with T antigen is even more striking (Fig. 3). Strong secondary immune responses to T antigen were found in the sera from all the RIR-Tag, RIP-Tag3 and Tag4 mice, comparable to that observed in normal mice. In comparison, two RIP-Tag2 mice (c,d) produced weakly immunoprecipitating antibodies, while two others (RIP-Tag 2a,b) did not respond. Thus it would appear that mice of the RIP-Tag2 lineage are tolerant to the SV40 large T antigen, whereas the other lineages are non-tolerant.

Pancreas histopathology

The frequent appearance of circulating autoantibodies to a protein which is expressed at detectable levels only in pancreatic β cells raises the possibility that this observation is associated with an immune response against the β cells. Four mice bearing autoantibodies have been analysed by treating cryostat sections of pancreas with histochemical stains such as haemotoxylin and eosin, or Giemsa. These analyses have revealed frequent abnormalities, including extensive vascularization, fibrosis, the presence of large and numerous lymph nodes, and clear indications of lymphocyte infiltration of the islets of Langerhans, with varying degrees of obvious disruption (Fig. 4). The islets contain small, atypical cells in the region normally composed primarily of β cells. These cells show histochemical staining patterns characteristic of lymphocytes. Similar cells are present in adjacent enlarged blood vessels or lymph nodes.

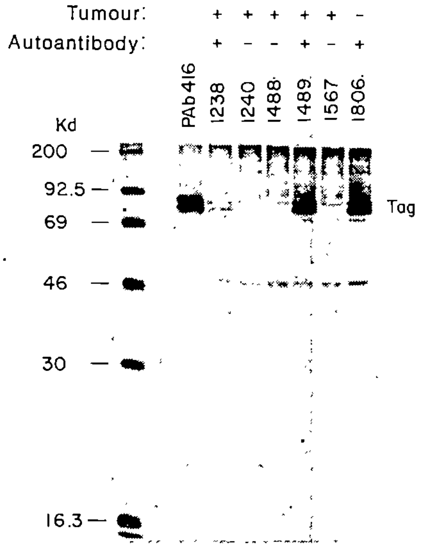

Fig. 2 Serum analysis of tumour and non-tumour bearing transgenic mice. The presence of autoantibodies to large T antigen in the sera of several tumour-bearing mice (1238, 1240, 1488, 1489, 1567) and one non tumour-bearing mouse (1806) of the RIP-Tag3 lineage was analysed by immunoprecipitation from ^{35}S-methionine-labelled COS cells as described (Fig. 1). Included in the analysis was the T-antigen-specific monoclonal antibody PAb416. The presence of pancreatic tumours was determined by microscopic analysis of the pancreas after killing individual mice. Blood for serum isolation was collected concurrently.

A spectrum of islet morphologies, ranging from virtually complete disruption to relative normality, is observed in the two autoimmune pancreases shown in Fig. 4. Multiple sections were taken through each pancreas so as to represent the entire tissue. At least 30 islets were evaluated for lymphocyte infiltration, which was found in 55% and 60% of the islets of the two mice. Neither of these mice had vascularized tumours, either visibly or histologically. The observed insulinitis is, therefore, almost certainly a response against the β cells in the islets of Langerhans. A mouse from the RIP-Tag3 lineage that was immunized with exogenous large T protein also showed lymphocyte infiltration in 80% of the pancreatic islets (Fig. 4). This suggests that the immune system is capable of both locating and responding to cells that synthesize large T, regardless of the route by which the immune response to T antigen was first initiated.

In tests of more than twenty mice from the tolerant RIP-Tag2 family we found no evidence of the abnormalities described

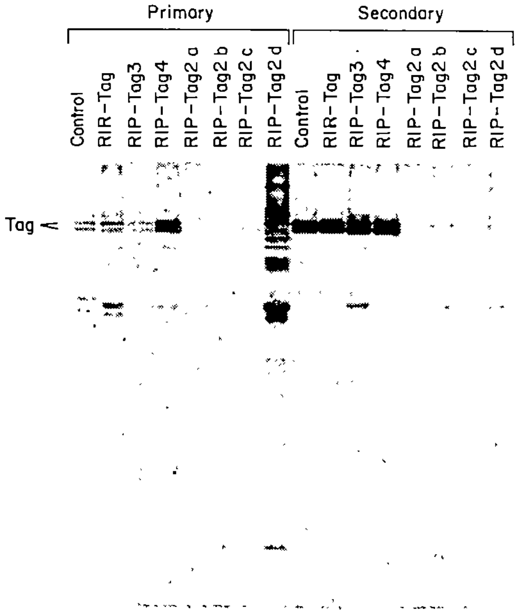

Fig. 3 Primary and secondary antibody responses of transgenic mice immunized with purified SV40 large T antigen. Depicted in the figure are the primary and secondary antibody responses of random individuals representing the non-transgenic control, RIR-Tag, RIP-Tag3 and RIP-Tag4 lineages, as well as the responses of four individual mice of the RIP-Tag2 (a–d) lineage. Four mice from each lineage were analysed (including the control). A typical result for each responding type is shown.

Methods. Immunoaffinity purified SV40 large T antigen (a gift from Y. Gluzman) was used as the immunogen. Mice (6–8 weeks old) of the RIP-Tag2, -Tag3, -Tag4 and RIR-Tag transgenic lineages were bled and screened for the presence of serum autoantibodies to T antigen. The mice were immunized (day 0) intraperitoneally (i.p.) with 10 µg of purified T antigen in complete Freunds adjuvant in a final volume of 0.2 ml. Fourteen days later the animals were boosted with a further 10 µg of antigen (in incomplete Freunds adjuvant) administered i.p. Mice were tail-bled on days 7 (primary response) and 21 (secondary response), serum isolated, and the antibody response to T antigen analysed by immunoprecipitation as described (Fig. 1). Included in the study as normal controls were mice of the B6D2F1/J strain.

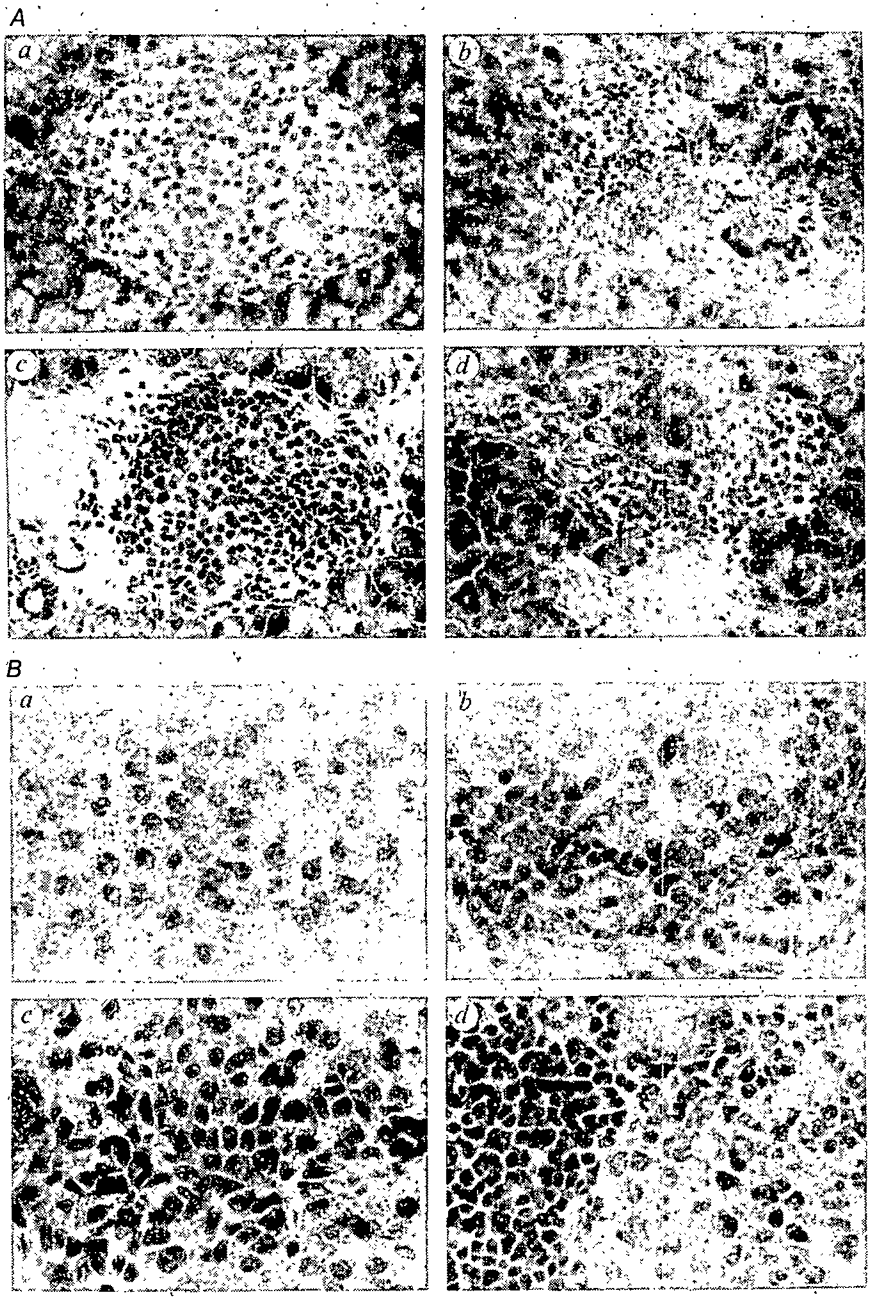

Fig. 4 Pancreas histopathology in mice producing antibodies against T antigen. Two mice bearing autoantibodies (2365 and 3217), and one with induced antibodies (1812) against SV40 large T antigen were analysed by cryostat sectioning of the pancreas followed by histochemical staining with hematoxylin and eosin. Mouse 2365, A RIP1-Tag3 mouse, was 8½ months old at the time of this analysis. The pancreas had β cell hyperplasia but no distinct tumours (either visibly or histologically). Mouse 3217, a RIP-Tag4 mouse, was 6 months old and did not have significant β cell hyperplasia or solid tumours, although enlarged lymph nodes were present. Mouse 1812 was immunized with 20 μg of purified SV40 large T protein at 6 weeks of age, and analysed at 11½ months of age. No tumours or hyperplasia were noted in mouse 1812. *A*, Characteristic morphology of pancreatic islets in these mice: *a*, normal, non-transgenic mouse islet; *b*, islet from mouse 1812, who was immunized with Tag; *c*, islet from mouse 2365; *d*, islet from mouse 3217, showing an adjacent aggregation of lymphocytes (other islets in this pancreas had large holes as seen in *c*). All magnifications were 160×. *B*, High magnification (400×) photos showing the interior regions of pancreatic islets: *a*, normal islet full of β cells; *b*, islet from mouse 1812, immunized with exogenous large T at a different location (i.p.); *c*, islet from mouse 2365; *d*, segment of a lymph node found within a hyperplastic islet in the pancrease of mouse 2365.

Methods. Pancreases were removed in phosphate buffered saline and infiltrated with 30% sucrose. The tissues were frozen in Lipshaw mounting media and sectioned on a cryostate microtome at 10 μm. The sections were stained with a standard histochemical procedure for haemotoxylin and eosin (Sigma).

above. Thus the immune response against the endocrine pancreas occurs only in lines of mice that are not tolerant to this transgenic β cell antigen.

Ontogeny of T antigen expression

The establishment of tolerance to self must require presentation (and hence expression) of self antigens, probably during late embryogenesis and early neonatal life. Mice from each of the families of transgenic mice harboring insulin-T antigen genes have been examined for expression of large T antigen by immunohistochemistry, using antibodies which specifically recognize this protein. An analysis of neonatal and adult mice from the tolerant RIP-Tag2 lineage, from the non-tolerant RIP-Tag3 and -Tag4 lineages and of normal non-transgenic mice is shown in Fig. 5. Large T is clearly expressed at high levels in RIP-Tag2 mice, both neonatally and in adults. Similar analyses of 22 mice in this family from the last week of embryogenesis throughout adulthood reveals that T antigen is expressed at high levels in β cells at all times.

In contrast, the non-tolerant lines do not show T antigen expression in neonatal mice, and even by ten weeks of age expression is heterogenous in the islets. Immunostained sections of 40 islets from four mice (10 weeks old) in the non-tolerant lineages showed that 0.0–3.3% of the cells in an islet had high levels of large T. This is in contrast to the RIP-Tag2 mice, where 35–100% of the cells in each islet section contain T antigen. It thus appears that, although the non-tolerant lines express large T specifically in the β cells (as do mice in the RIP-Tag2 family), there is a delay in the onset of expression.

A model for self-tolerance and autoimmunity

We have shown that the delayed onset of expression of a hybrid insulin/SV40 T antigen gene in several lines of transgenic mice results in failure to establish self-tolerance and, consequently, production of antibodies specific for large T antigen. We propose that similar molecular mechanisms may be involved in the aetiology of diseases of apparent autoimmune origin, in particular insulin-dependent diabetes.

The expression of a number of intact and hybrid genes has been shown to be tissue-specific in transgenic mice[10]. However, the transgenes often have unusual characteristics of expression when compared to the corresponding endogenous genes, includ-

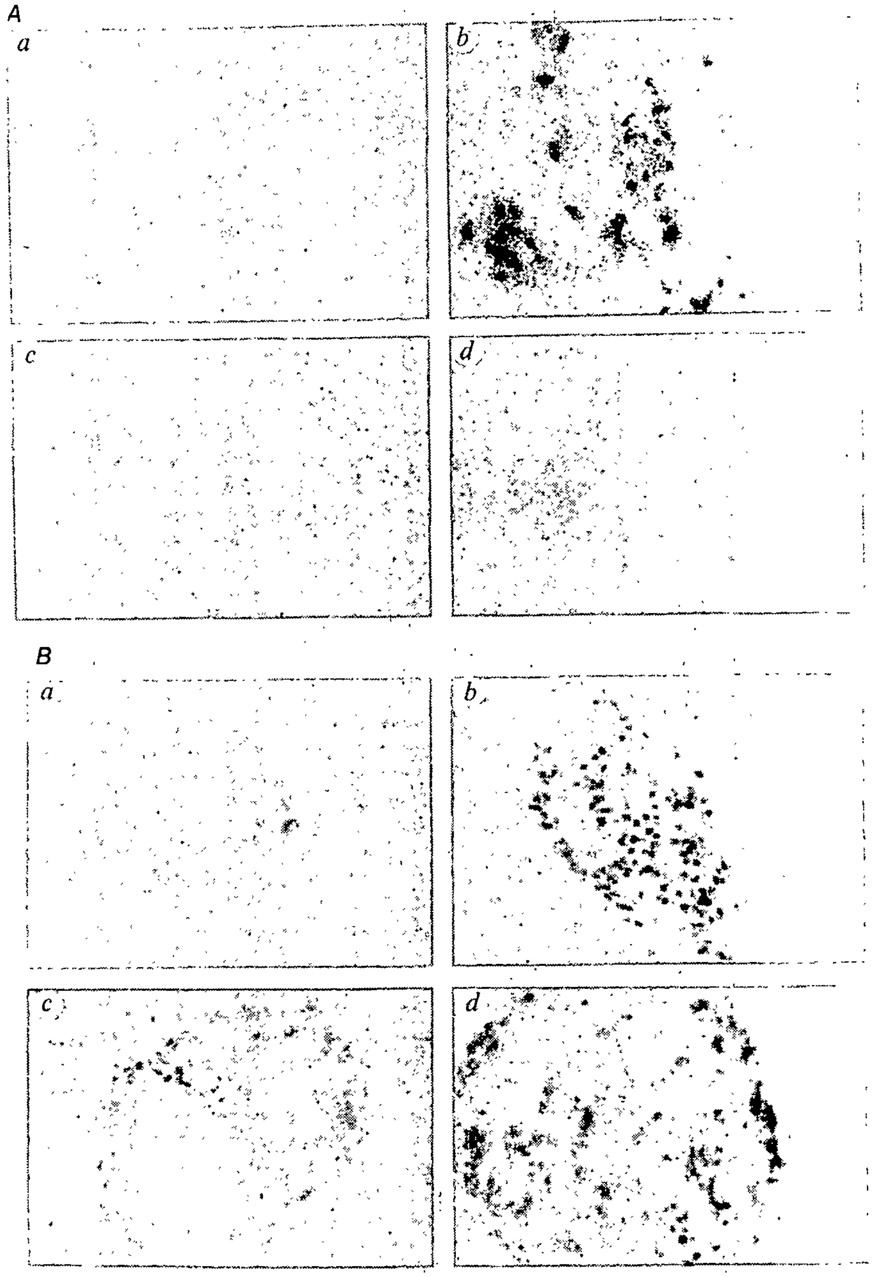

Fig. 5 Ontogeny of expression of T antigen in the pancreas. Neonatal and adult pancreases from normal mice and from RIP-Tag2, -Tag3, and -Tag4 mice were analysed for the presence of T antigen by immunostaining. *A*, analysis of neonatal mice: *a*, normal; *b*, RIP-Tag2; *c*, RIP-Tag3; d, RIP-Tag4. The primary polyclonal anti-Tag antibody was visualized using an ^{125}I-labelled second antibody and autoradiography. This method allows identification of very low levels of T antigen expression. Note that no expression is detectable in the RIP-Tag3 and -Tag4 lineages. The grains in the RIP-Tag2 pancreas (*b*) show characteristic nuclear localization for large T. *B*, analysis of 10-week-old mice from the same lineages, in the same order, using immunoperoxidase visualization of the antibody-antigen complex. The magnification is 200× in *A*, and 160× in *B*.

Methods. Neonatal mice were killed by cervical dislocation and dissected in phosphate buffered saline. The pancreas were removed into a fixative solution of 4% paraformaldehyde buffered to *p*H 7.4 with 0.1 M sodium phosphate buffer and postfixed for 1 h. The fixed tissues were infiltrated with 30% sucrose, mounted in Lipshaw M1 medium, sectioned in a cryostat microtome, and the sections mounted on gelatin-coated slides. Adult mice were perfused through the heart with the fixative solution, the pancreas was removed and postfixed for 1 h. Further processing was carried out as described above. The sections were washed in Tris-buffered saline (TBS, 0.1 M Tris, *p*H 7.5 and 0.9% NaCl), exposed first to 0.25% Triton X-100 in TBS for 15 min, then to goat serum diluted 1:30 in TBS for 30 min, and incubated overnight with a rabbit polyclonal antibody to T-antigen (a gift of David Lane). After the primary antibody treatment (1:500) the sections were treated for immunoperoxidase staining as described[24]. The sections were washed with TBS, incubated with a 1:50 dilution of goat anti-rabbit IgG (Miles Scientific) for 30 min, rinsed with 1% goat serum in TBS, and then exposed to a 1:100 dilution of rabbit peroxidase anti-peroxidase (gift of G. Teitelman) for 30 min. Bound peroxidase was visualized by reaction with 0.25 mg ml^{-1} of 3,3′-diaminobenzidine (DAB, Aldrich), 3 ml^{-1} of nickel sulphate (Sigma) and 0.01% hydrogen peroxide. After the DAB reaction the sections were dehydrated through graded alcohols and mounted with Permount. For autoradiography, the sections were washed with 1% goat serum in TBS after primary antibody reaction (1:5,000), then incubated for 30 min in a 1:50 dilution of donkey anti-rabbit IgG labelled with ^{125}I (specific activity of 5–20 μCi μg^{-1} Amersham). The sections were washed, air-dried overnight and dipped in Ilford L-4 photographic emulsion (Polysciences) diluted 1:1 in water. The slides were air-dried and placed in a light-tight box containing a drying agent at 4 °C. After 3 weeks the sections were developed for 2 min at 16–17 °C in Kodak D-19 developer, rinsed in water, fixed in Kodak Ektaflo (diluted 1:3 in water) for 8 minutes at 4 °C, and then dehydrated through graded alcohols and mounted with Permount.

ing failure to express at all. It has been suggested that transgenes integrated into 'transcriptionally silent' regions of chromatin (for example, heterochromatin) may be incapable of expression[10,11]. This type of nonspecific dominant repression, which might be termed a position effect, could be the basis for the differential patterns of T antigen expression in the RIP-Tag3 and -Tag4, and RIR-Tag lineages when compared to the RIP-Tag2 mice. The subsequent tissue-specific expression of T antigen in RIP-Tag3, -Tag4, and RIR-Tag mice may result from the relaxation of this non-specific repression as the cells age; eventually the *β* cell specific trans-acting factors are allowed access to the *β* cell specific regulatory region which resides in this inaccessible location in the genome.

Why are these lines of transgenic mice heritably non-tolerant to large T antigen? It is well established that neonatal mice are readily tolerized to foreign antigens[1–3], indicating that this stage of development is critical for the ability of a maturing immune system to establish a mechanism of self-discrimination. In the RIP-Tag2 mice, embryonic/neonatal expression of large T antigen in the pancreas is apparently sufficient to establish T antigen-specific tolerance. As presentation of an antigen to the immune system during the period of self-learning is thought to require its circulation, it will be important to determine serum levels of T antigen shortly after birth. RIP-Tag2 mice can produce antibodies that weakly immunoprecipitate T antigen after immunization with large T protein, suggesting that the B lymphocytes capable of recognizing T antigen have not been completely depleted. This interpretation is consistent with the inducibility of B cell responses to proteins normally recognized as self, as discussed recently by Mitchison[12]. An argument can thus be made that the tolerance to large T resides in another compartment of the immune system, perhaps in that of the T helper or

T suppressor cells. Because of delayed developmental expression, the mechanism which produces self-tolerance to T antigen is not effective in RIP-Tag3, -Tag4 and RIR-Tag mice. The availability of these transgenic mouse lineages will allow further experimentation to elucidate the cellular basis of tolerance to this transgenic self-antigen.

The results presented here also motivate an analysis of the time course of the autoimmune response in individual mice. It is possible that the immune response will persist in some mice, and die out in others, which may in turn affect both the rate of tumour formation and the extent of islet destruction. There are two competing processes in the autoimmune mice: β cell proliferation and β cell destruction. This may sometimes result in a balance, which could be relevant to strategies for counteracting the slow β cell destruction in type 1 diabetes by inducing slow β cell proliferation.

The observation that delayed expression of β-cell specific large T antigen results in autoantibody (and presumably cellular) responses is relevant to a number of autoimmune diseases such as insulin-dependent diabetes. Many factors may lead to the initiation of IDDM development, but it would appear that the destruction of pancreatic β-cells is immune-mediated[5,13]. Autoantibodies to cytoplasmic[14] and cell-surface[15] antigens of islets are prevalent, and lymphocyte infiltration of the pancreatic islets has been documented[16,17]. It is notable that in type I diabetes of humans and rats, an islet cell protein of M_r 64K appears to act as a major antigenic target during the development of the disease. Autoantibodies to the 64K protein appear in the sera of humans and IDDM-susceptible rat strains well before clinical manifestation of the disease are apparent[18,19]. It is important to establish the developmental pattern of expression of the 64K protein and other major antigenic targets in normal and IDDM-susceptible individuals. Perhaps the delayed onset of expression of a β cell specific gene, such as that encoding the 64K autoantigen, results in failure to establish self-tolerance to its gene product. The consequence of this non-tolerance might be the induction of humoral and/or cellular autoimmune responses when the 'foreign' β cell antigen eventually appears, resulting in β cell destruction and the development of diabetes.

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LETTERS TO NATURE

A model for collimated outflows in molecular clouds and the case of HH 7–11

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Collimated outflows of high-velocity gas, with typical scale lengths of 0.01–0.2 pc, are often observed to be associated with linear chains of optical emission knots[1], sometimes connected by faint nebulosities. The flows have been ascribed to the interaction between an energetic wind ejected by a young stellar object and circumstellar material. However, none of the acceleration and collimation mechanisms proposed so far can easily account for the most peculiar properties of the outflows, namely the almost constant spacing between the knots, their radial motion and the abrupt velocity changes along the flow, well illustrated by the prototype we use for our modelling, HH 7–11. We suggest here a new interpretation of the phenomenon, based on flows in a channel of variable cross-sectional area due to Kelvin–Helmholtz instabilities between the flow and the ambient medium; and present solutions of the Mach number equation for such a channel, which possess multiple critical points and shocks identified with the observed optical knots.

Linear chains of optical emission line knots aligned with infrared sources embedded in dark clouds possess recession velocities which are interpreted as due to outflow from the infrared source. These chains often lie along the axis of high-velocity bipolar CO outflows, which have been observed in association with molecular clouds[2]. Although such molecular flows, in general, have poor collimation (the opening angle is <20° in only a few sources), new data on optical jets show much lower values for opening angles (~5°–10°). These data also trace the flow very close (1–2 arc s) to their driving star; thus collimation must take place on distances $\lesssim$100 AU from the source. The wind-flow model that we propose is motivated by remarkable structural similarities between the latter well-collimated outflows in molecular clouds and jets from active galactic nuclei and quasars[3], which suggest that similar hydrodynamic phenomena may occur in objects with widely different length scales (see Fig. 1). To illustrate our proposal we shall focus our attention on the chain of Herbig-Haro objects HH 7–11, which seem to be excited by a wind flowing out of the infrared source

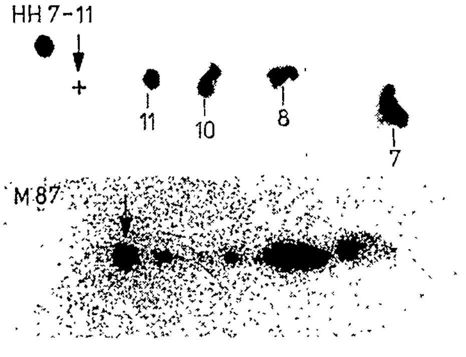

Fig. 1 The chain of optical knots HH 7-11. The arrow indicates the position of the driving infrared source. A similar morphology is observed in the optical jet of the elliptical galaxy M87.

HH 7-11 IR in the NGC1333 region. Our choice has to do with the remarkably uniform spacing of these objects, which may be a prototype for molecular outflow sources.

The NGC1333 complex is estimated to be at a distance of ~500 pc from Earth[4]. The molecular cloud was mapped[5] in the (J, K) = (1, 1) line of NH_3, and the southern condensation is estimated to have mass ~230 $M_\odot$. Bipolar CO gas flow associated with a low-luminosity infrared source in the densest part of the cloud has been discovered[6] in the vicinity of HH 7-11. The peak intensities of blueshifted and redshifted wings are separated by 1.05 arc min (~0.20 pc), the total mass in high-velocity gas is estimated to be 4.2 $M_\odot$ (1.3 $M_\odot$ in blueshifted gas), and the total kinetic energy is 7×10^{45} ergs. A map of the region in the CS(J = 3 → 2)line[7] gives evidence for an elongated structure of high-density gas through the source of outflow, orthogonal to the separation of the 'red' and 'blue' high-velocity CO components. Measured[4] radial velocities range from −16 to −133 km s^{-1}, with an uncertainty ±15 km s^{-1}, for the chain of aligned objects HH 7, 8, 10 and 11, respectively, with the proper motion μ = 3.5 arc s per 100 yr for HH 11 (ref. 8), giving a transverse velocity v_t = 83 km s^{-1} at an angle ψ = 154° to the line of sight, at the source distance. The infrared source HH7-11 IR (ref. 9), is an exceptionally red object (K-L > 2.0), with an estimated lower limit to the bolometric luminosity of ~60.8 $L_\odot$ (ref. 10). This infrared source "has surely somehow ejected the matter exciting the chain HH 7-11"[10]. The parameters of the HH 7-11 chain are summarized in Table 1.

For modelling the flow over the scale length of the HH 7-11 chain, we assume the system to consist of a central condensation, plus an envelope of negligible mass. Indeed, the measured colour excess[11] towards HH 11 and HH 7, together with an estimate for the mean extinction in the galactic plane[12] and the extinction versus column density relation[13], allow us to estimate that both the mass in the spherical shell between HH 11 and HH 7 and the mass in the cloud outside HH 7 are essentially negligible when compared with the total cloud mass. We shall then assume that a central mass is surrounded by a circumstellar disk in which two narrow channels are formed, and through which the collimated stellar wind flows.

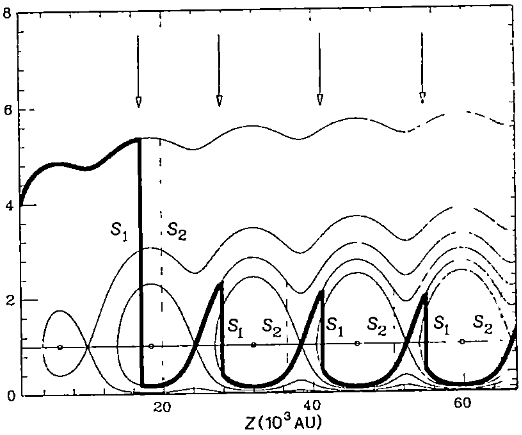

Fig. 2 Behaviour of the flow Mach number M with distance Z. The complete topology of solutions is plotted: the actual solution for the chain HH 7-11 is marked by the heavy line. The pairs of shocks S_1 and S_2 connecting different solutions are shown; the arrows indicate the positions of those shocks we associate with the objects (from the left) HH 11, HH 10, HH 8 and HH 7, respectively. The plot is given for an isothermal flow with: $m = 20\ M_\odot$, $T_0 = 2\times10^4$ K, $\varepsilon_0 = 0.45$, $M_0 = 4$ and λ = 14,000 AU.

The pressure of a circumstellar disk may be important in collimating the jet. For our source, detection[7] of the CS transition J = 3 → 2 implies a density $\gtrsim 1.6\times10^6$ cm^{-3}, which suggests that the jet could be pressure confined; estimates of the density in other similar objects, such as L 1551 IRS5, give values[14] ~4×10^6 cm^{-3}. Thus, we shall assume that at some base radius, say $z_0 = 10^3$ AU, the wind is already supersonic and collimated with a uniform temperature $T_0 \approx 10^4$–10^5 K, corresponding to a sound speed $c_s \approx$ 16–50 km s^{-1}. A flow thermally confined by the external medium is probably Kelvin-Helmholtz unstable. As studied in detail in the case of extragalactic jets[15], a beam develops unstable pinching modes, whichever is its Mach number, which lead to compressions and rarefactions of the gas periodically distributed (depending on the perturbation wavelength λ) along the jet. Subsequent propagation along the channel will then be governed by the equations of mass and momentum conservation, that in a quasi-two-dimensional approximation[16] can be combined into a single equation for the Mach number $M = v/c_s$ (with $Z = z/z_0$),

$$\frac{M^2-1}{2M^2}\frac{dM^2}{dZ}=\frac{d\ln A(Z)}{dZ}-\frac{Gm}{2c_s^2Z^2z_0} \qquad (1)$$

where $A(Z)$ is the variable cross-sectional area of the channel, $A(Z) = Z^2[1+\varepsilon_0\sin(2\pi Zz_0/\lambda)]^2$. In deriving these expressions, we have adopted spherical symmetry, that is the beam is assumed to be conical with fixed opening angle, modulated by a pinching perturbation with wavelength λ, and amplitude ε_0 at $Z = 1$. The quasi-two-dimensional approximation used here is only valid for causally connected streamlines, that is, for the flow along an infinitesimal flow tube lying on the axis of the beam. As is usual in such quasi-two-dimensional treatments[17,18], we adopt the rate of geometric variation of the macroscopic flow channel as an appropriate measure of the cross-sectional areal variation of the elemental flow tube along the channel axis. In the present case, these areal variations imply periodic subtraction (for contraction) or input (for expansion) of momentum to the flow, which can lead to the formation of regularly-spaced standing shocks. As heating of gas is expected in a shock, we propose that a sequence of such shocks can be associated with the observed HH chain. The limits of this interpretation depend on our 'slender tube' approximation; hence, we cannot account for the possible effects of oblique shocks (which are connected with the interaction of the beam surface with the external medium). Thus, strictly speaking, we cannot use our very simple model

Table 1 Distances from HH 7-11 IR and velocities of the knots of the chain HH 7-11

	θ (″)	Distance (AU)	Velocity (km s^{-1})
HH 11	12.4	12,500	168
HH 10	26.6	26,500	33
HH 8	40.2	40,500	50
HH 7	62.1	63,000	67

Table 2 Positions of shocks and velocities for the following parameters (assuming $\lambda = 14{,}000$ AU)

Case a		Case b		Case c	
Shock position (AU)	Velocity (km s^{-1})	Shock position (AU)	Velocity (km s^{-1})	Shock position (AU)	Velocity (km s^{-1})
16,990	126	16,370	317	17,250	233
19,810	126	20,210	317	20,120	233
27,750	55	27,430	120	28,730	55
36,490	55	36,730	120	35,930	55
41,170	51	40,480	110	42,010	46
50,750	51	51,230	110	50,290	46
54,910	47	54,250	104	55,610	41
64,170	47	65,350	104	64,490	41

Case a: $m = 20\ M_{\odot}$, $T_0 = 2\times10^4$ K, $\varepsilon_0 = 0.45$, $M_0 = 4$, $\gamma = 1$.
Case b: $m = 100\ M_{\odot}$, $T_0 = 1\times10^5$ K, $\varepsilon_0 = 0.5$, $M_0 = 5$, $\gamma = 1$.
Case c: $m = 20\ M_{\odot}$, $T_0 = 1\times10^5$ K, $\varepsilon_0 = 0.3$, $M_0 = 4$, $\gamma = 4/3$.

to directly infer phenomena on resolved transverse dimensions, for which complete two-dimensional numerical simulations are required.

In principle, we ought to take into account the velocity of the perturbations along the surface of the jet; however, for the fastest growing perturbations this velocity is quite small so that the areal distortions can be considered localized without loss of generality[19]. Solutions of equation (1) have been found for the case of HH 7-11, assuming $\lambda \approx 14{,}000$ AU, the initial $M(Z = 1) = M_0 > 1$, and $\varepsilon_0(<1$ always) to be a free parameter of the solutions. Figure 2 shows a plot of a representative solution for a central mass $m = 20\ M_{\odot}$, $T_0 = 2\times10^4\ K$, $\varepsilon_0 = 0.45$. The main feature of interest is that with $A(Z)$ given as above, we find multiple critical points and shocked solutions. The position of each shock is determined by the Rankine-Hugoniot condition for conservation of mass and momentum fluxes across the discontinuity for an isothermal gas, $M_1 M_2 = 1$, where M_1 and M_2 are the Mach numbers upstream and downstream from the shock, respectively: every critical solution can be connected with the following one by two shock transitions (with the same velocity) at the positions S_1 and S_2. In Table 2 (case a), we report the position of the shocks with the corresponding upstream flow velocity: we see that the first of each pair of shocks (S_1) can be tentatively associated with the observed morphology; in particular, we associate the first fast moving shock with HH 11, and the slower shocks with HH 10, HH 8 and HH 7, respectively. The essential question is then whether these shocks actually form; the answer to this crucial question can only be found by a time-dependent treatment of the problem because the present stationary solutions select only the positions where the shocks can form.

The dependence of the results on the value of the central mass and on the assumption of uniform temperature flow was explored by modelling the cases of an isothermal flow with $m = 100\ M_{\odot}$, $T_0 = 10^5$ K, $\varepsilon_0 = 0.60$ (case b) and a polytropic flow ($\gamma = 4/3$) with $m = 20\ M_{\odot}$, $T_0 = 10^5$ K and $\varepsilon_0 = 0.30$ (case c). The position of the shocks is almost the same in the three cases and again fits the position of the HH objects (Table 2). However, a larger central mass requires a higher temperature to accelerate the flow, and also a larger beam areal disturbance amplitude to produce the shocks (case b): therefore a central mass $\gg 20\ M_{\odot}$ implies velocities larger than those observed. A slight departure from isothermal conditions leads to velocities similar to the isothermal case, but for lower amplitudes of perturbations, that is, shock formation is obtained under less restrictive conditions in polytropic flows (case c). Furthermore, if a diffuse mass comparable or larger than the central one is present in the outflow region, our results must be recalculated because the flow can be severely affected by a spatially distributed mass: for specific radial mass distribution laws, no stationary outflow solutions may exist[19].

Our interpretation of the HH 7-11 chain as arising from the interaction of a collimated outflow with dense circumstellar material agrees with well-established properties of the HH nebulae, whose ionization and excitation appear to be dominated by collisional processes in shock waves with shock velocities ~100 km s^{-1}. Furthermore, proper motion data of HH objects, and their alignment with the infrared source in several cases, suggest that the supersonic flows responsible for excitation are highly collimated[20]. New observational data[21] show several bright HH objects to be associated with a continuous jet. For the HH 7-11 chain, a diffuse spatially extended emission in the S_1 line of H_2 was detected[22], and interpreted as arising from the same low-velocity shock that causes visible emission. Similar results[23] were obtained for the same regions in the $v = 1 \rightarrow 0$ Q-branch line of H_2. In addition, weak Hα emission has been recently detected[24] between the knots of the HH 7-11 chain. These data are consistent with a model in which HH knots are powered by highly collimated quasi-continuous flows, the knots themselves marking the location of increased energy dissipation in the shock fronts occurring along the flows. The observation[25] of abrupt velocity changes over very small spatial elements in the bipolar gas flow surrounding L1551 IRS5 supports our model.

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Cometary magnitude distribution and the fading of comets

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Long-period comets are, on average, more luminous than short-period ones. This paper considers the recent observations of the nucleus of comet Halley, in conjunction with the results of the modelling of the accretion process that is most probably responsible for cometary origin. It now seems that simple physical processes such as a rounding off and removal and/or settling of bumps and prominences protruding from the nucleus plus a change in the percentage of the nucleus surface that is active could account for the fading that occurs just after jovian cometary capture.

A series of observations of the apparent magnitude, m, of a specific comet fit well a formula of the type

$$m = H_{10} + 2.5\, n \log r + 5 \log \Delta \qquad (1)$$

where r and Δ are the comet-Sun and comet-Earth distances and H_{10}, the absolute magnitude, is a product of the fitting in the specific case where n is forced to be 4.0. A file of H_{10} values has been produced using data published by Vsekhsvyatskii[1-5] and Vsekhsvyatskii and Il'ichishina[6]. The accuracy of specific H_{10} values is enhanced for long-period comets by the fact that 60% of this group have perihelion distances, q, <1 AU and so H_{10} can be obtained by interpolation using equation (1). Of known long-period comets, 89% have $q < 2.0$ AU (ref. 7). Only 19% of short-period comets have $q < 1.0$ AU (85% have $q < 2.0$ AU) but this is partially compensated for by the fact that most of these have been seen more than once, leading to an increase in the accuracy of H_{10}. A plot of the logarithm of the number of comets, N, observed up to the present to be brighter than a specific absolute magnitude, H_{10}, against that magnitude (Fig. 1) produces a cumulative curve where

$$N = b a^{H_{10}} \qquad (2)$$

to the right of a knee value. To the left of that value the data are greatly affected by observational selection because of Earth-based observers missing faint comets.

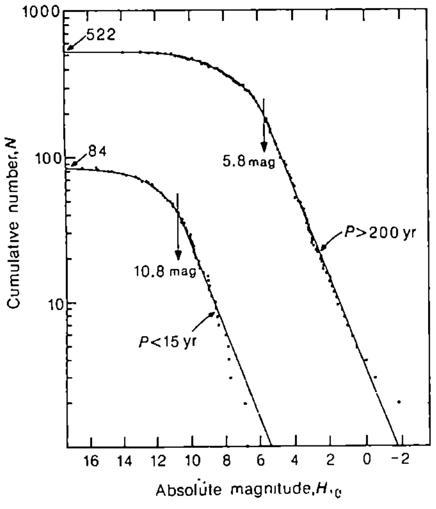

Fig. 1 The cumulative number, on a logarithmic scale, of short period ($P < 15$ yr) and long period ($P > 200$ yr) comets as a function of their absolute magnitude, H_{10} (see equation (1)). The line through the $P > 200$ yr data has been obtained by linear regression fitting and the line through the $P < 15$ yr has been purposely drawn such that its gradient is the same as that of the long period line. The fit to these data is good. The data break away from linearity at 5.8 mag and 10.8 mag respectively. Mankind has so far recorded 522 long-period and 84 short-period comets sufficiently well to obtain good absolute magnitude values.

These data have been divided crudely into two groups, long period comets (with period, $P > 200$ yr) which have been seen only once and have probably been recently captured from the Oort/Öpik cloud and are evolutionarily 'new', and short period comets (period, $P < 15$ yr) which have been captured into the inner solar system by Jupiter, have a low orbital inclination, a median period of ~7 yr and are evolutionarily 'old'—being smaller and less massive than they originally were.

Four things are immediately noteworthy in Fig. 1. The right hand, linear portions of both curves essentially have the same gradient, being represented by

$$\log N = 0.549 + 0.301\, H_{10} \quad (P > 200\ \text{yr}) \qquad (3)$$

$$\log N = -1.625 + 0.301\, H_{10} \quad (P < 15\ \text{yr}) \qquad (4)$$

The knee in the long period curve occurs at 5.8 mag whereas the short period knee is at 10.8 mag. The vertical separation of the two right hand portions is equivalent to a factor of 150. This contrasts with the fact that in recorded scientific history we have noted 522 long period comets and 84 short period comets, a ratio of 6.2:1.

How do we explain these observations? Let us first move a step beyond the absolute magnitude. For a well-behaved periodic middle-aged comet such as Halley it is reasonable to assume that the cometary luminosity is proportional to the surface area of the nucleus[8,9] and thus produce[10,11] formulae of the type

$$\log R = 1.842 - 0.2\, H_{10} \qquad (5)$$

and

$$\log M = 20.845 - 0.6\, H_{10} \qquad (6)$$

where R(km) is the equivalent radius of the nucleus (the radius of a sphere with the same surface area) and M(g) is the mass of the nucleus. The only cometary nucleus that has been imaged to date is that of Halley. It is relatively smooth and potato shaped, and on March 14 1986 was emitting gas and dust profusely from about 8% of its surface area[12]. (The adjective 'well-behaved' is applied to Halley because observations of the last thirty apparitions have indicated that its H_{10} value at each return[13] has remained reasonably constant at $\sim 5.5 \pm 0.7$.) If it is assumed that a typical comet loses a layer of constant thickness from its active regions each time it passes the Sun and that the ratio between the active and inactive area remains constant, the decay can be easily modelled[10]. The process is slow and as the comet ages through 10, 20, 30, 40 and 50% of its inner solar system life its mass drops to 73, 51, 34, 22 and 13 percent respectively of its initial value and its absolute magnitude, which on capture was H, becomes $H + 0.23$, $H + 0.48$, $H + 0.77$, $H + 1.1$ and $H + 1.5$ respectively. It can thus be concluded that a capture and decay, which keeps constant both the general shape and the percentage of the surface area that is active, changes the absolute magnitude by only a small amount. The presently accepted mechanism[14-16] for transferring a long period comet into a short period orbit, a mechanism which requires only a few near tangential close passages of the jovian orbit, will, if the above assumptions are correct, do little to change the absolute magnitude of a comet. So there is no *a priori* reason why a specific group of long period comets should have a median absolute magnitude that differs significantly from the group of short period comets that they are perturbed into.

Where have we gone wrong? Why do comets fade between their long and short period existence? Previous suggestions relied on chemistry, having new long period comets coated with some supervolatiles. After a few close passages of the Sun these had been lost leaving behind the more mundane mixture of H_2O, CO_2, NH_3, CH_4 and so on.

But why not simply rely on physics? The magnitude distribution index, a, in equation 1, is 2.0 ± 0.2 (see equations (3) and (4)). Using equation (6) this can be converted to a mass distribution index, s, of 1.75 ± 0.1. (This index is defined such that the

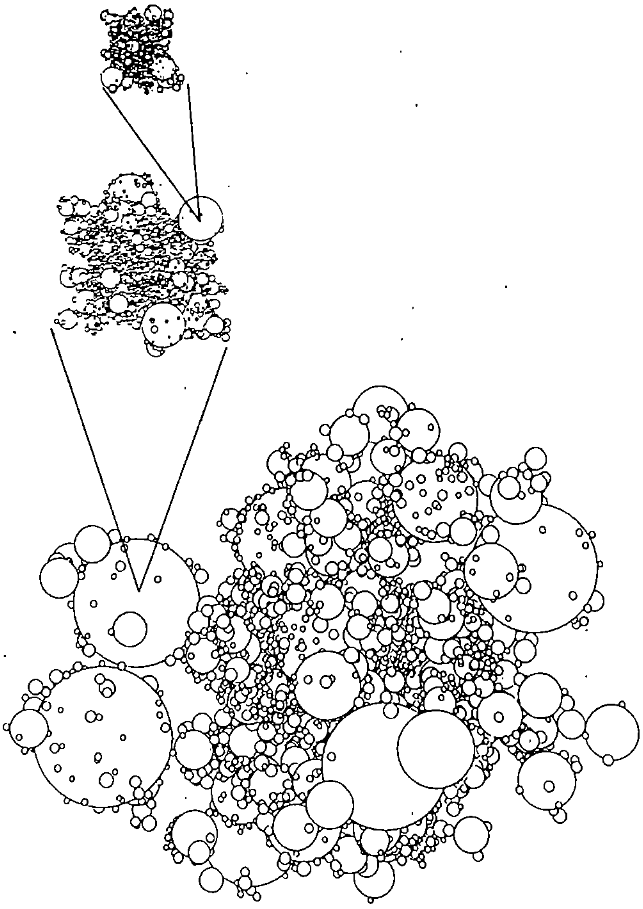

Fig. 2 A fractal model of a newly accreted cometary nucleus. This is a hierarchical porous, low density structure which is, as shown, self similar over a vast range of scale lengths from kilometric down to micrometric.

number of comets with mass greater than M is proportional to $M^{(1-s)}$.) Such a low mass distribution index is indicative[17] of the fact that accretion, as opposed to fragmentation, is the main agent responsible for formation. The resultant comet will have a fractal form[18,19] such that its hierarchical, porous structure will be nearly self similar over a vast range of scale lengths from kilometric down to micrometric. The fractal model of a newly accreted comet is illustrated in Fig. 2. The hummocky nature of this model differs from the rather smooth shape presented by the images of Halley. The first few solar close approaches of a new comet could result in a general rounding off of its features and a sharp decrease in the surface area to mass ratio. As luminosity has been assumed to be proportional to surface area this could result in a considerable change in the absolute magnitude. A sharp drop in surface area by a factor of say 2.5 changes the magnitude by 1.

Only 8% of the surface of Halley (a slowly rotating nucleus with a spin period of about 52 h) is active at any one time. Maybe a new comet has not had time to build up choking surface dust regions and thus all the sunward face of the nucleus, about 50% of its total area, is actively emitting gas and dust. The ratio 50/8 accounts for another 2 magnitudes.

Both these proposed effects change the magnitude by a total of say ΔH and equation (6), for new as opposed to middle-aged comets, becomes $\log M = (20.845 - 0.6\,\Delta H) - 0.6\,H_{10}$. This is equivalent to sliding the $P > 200$ yr curve in Fig. 1 to the left by ΔH and thus considerably decreasing the difference between the observed numbers of long and short period comets. The vertical separation between the two right hand portions of the cumulative curves (see Fig. 1) would then be $10^{(2.174-0.301\Delta H)}$. So a ΔH of 3 would change this factor from 150 to 19.

Returning to the other three noteworthy points in Fig. 1, the similarity in the gradients of the two curves is not at all surprising[20] if it is assumed that decay is equivalent to the loss of a constant thickness of a constant percentage of the nucleus surface at each apparition as opposed to a constant percentage of the mass. The observational bias inherent in cometary observation does not markedly affect the gradients of these two curves. Dividing the $P < 200$ yr comets into specific period groups leads to a set of a values scattered randomly about the mean value given above. Dividing the $P > 200$ yr comets into groups with differing perihelion distances again leads to a set of a values randomly scattered about the mean (see Hughes[21]).

The difference between the two knee positions, 10.8 mag for short-period comets and 5.8 mag for long-period comets, is equivalent to a factor of 100 in brightness. The discussion above enables us to change this 5 mag difference to $(5 - \Delta H)$ and a ΔH of 3 gives a factor of 6 in brightness. This can easily be accounted for by the differences in the probability of detection[22] between the two groups. The fact that we see a long-period comet only once and a short-period comet returns, usually along the ecliptic, on average every 7 yr underlines this difference.

We are still left with the problem of calculating the actual ratio between the number of long-period and short-period comets that enter a specific sun-centred volume of the solar system and have masses greater than a specific mass. Both the differences in observational probability and the differences in the absolute magnitude-mass relationships have to be taken into account. From Fig. 1 we can conclude that this ratio is $\leqslant 150$ but is more likely to be in the region of 20. The fact that the apparent value of this ratio is 6.2 means that many long-period comets fly past Earth undetected.

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Study of particle motion in concentrated dispersions by tracer diffusion

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Monodisperse spherical colloidal particles can be used to simulate molecular motions and, in particular, those which occur in the regions of phase transitions such as melting and freezing. In nonaqueous media the behaviour of sterically stabilized particles in near to that of 'hard-spheres'. The use of systems in which the majority of the particles are refractive index matched to the dispersion medium but where a few particles are added of the same

size and surface chemistry but of different refractive index allows the development of tracer particle measurements using photon correlation spectroscopy. Thus it becomes possible to examine particle motion in dense systems. Here we use this novel technique to determine particle self-diffusion coefficients over a wide range of volume fractions and into the 'frozen' state.

Studies on the physics of the liquid state and the phase transitions between liquids and solids have been hampered by the difficulties of labelling molecules in order to study their diffusional motion. However, one of the major developments in the field of colloid science over the past few decades has been the emergence of synthetic methods for the preparation of spherical polymer particles with a very narrow size distribution, that is, polymer latices or polymer colloids[1]. By the use of these particulate systems it has become possible to study the behaviour of dispersions of high volume fraction by small-angle neutron scattering[2] and light diffraction[3]. These systems, which are of interest in their own right for the study of particle-particle interactions in colloidal dispersions, also simulate the 'liquid state' and at volume fractions in the region of 0.5 enable the liquid-solid phase transitions for 'hard spheres' to be observed[4,5].

Although light forms an excellent probe for the examination of dilute strongly interacting systems[6] the interpretation of results from concentrated aqueous dispersions is complicated by multiple scattering because of the large difference between the refractive index of the particle and the medium. With aqueous based polymer latices little opportunity exists to vary the refractive index of the dispersion medium without changing the colloidal properties of the system. The development of methods for forming polymer latices in nonaqueous media[7], however, introduces wide possibilities in the choice of the dispersion medium. In earlier studies refractive index matching between particles and the medium was achieved using a mixture of dodecane and carbon disulphide[8,9]; matching was achieved over a narrow 'window' of wavelength and temperature although some swelling occurred on addition of carbon disulphide[10]. The particles used were composed of poly(methylmethacrylate) (PMMA) cores with chains of poly(12-hydroxystearic acid) chemically grafted to the core. Particles of this type are said to be sterically stabilized, that is, they interact with a short range repulsive potential essentially as 'hard spheres'[5,7].

The possibility of refractive index matching leads immediately to the prospect of developing 'tracer' methods for the examination of particle motion in dispersions using photon correlation spectroscopy and its feasibility has been demonstrated by Kops-Werkhoven *et al.*[11,12], using dispersions of silica particles in cyclohexane. These authors exploited the heterogeneity of the silica particles and hence their variation in refractive index as a function of temperature to obtain a few results for particulate diffusion at high volume fractions.

The technique is, however, capable of considerable versatility by a suitable choice of particles and medium. Moreover, by the correct choice of particle size both 'short-time' and 'long-time' diffusion coefficients can be determined. In this communication we report the use of the photon correlation technique to study the diffusion of tracer particles of PMMA in a wide range of volume fractions of polyvinyl acetate (PVA); both particles were stabilized by poly(12-hydroxystearic acid). The PVA particles had a refractive index of 1.471 and could be precisely optically matched using a mixture of *cis*-decalin (refractive index 1.481) and *trans*-decalin (1.469). PMMA particles of refractive index 1.483 were added in a ratio of 1:43 PVA particles. The hydrodynamic radius of the PMMA particles was 83 ± 2 nm and that of the PVA particles, 85 ± 2 nm. It was therefore assumed, as the same stabilizer was used and the particle sizes were very close, that the repulsive interactions between the particles were identical.

With this system it has proved possible to carry out tracer diffusion studies over a wide range of scattering vectors (**K**), correlation delay times (τ) and volume fractions. The volume fractions examined ranged from dilute, where essentially free Brownian motion occurred, through the intermediate 'liquid-like' region, up to 0.54 just above the suggested onset of freezing for a hard-sphere system. As the volume fraction increases and particle-particle interactive contacts increase the diffusional motion of the particles can be separated into self-diffusion and collective diffusion[13]. The tracer technique measures the self-diffusion of particles and further, by making measurements at short correlation times, such that the particle has only moved a distance corresponding to a small fraction of a particle radius, the short-time diffusion coefficient can be obtained. On a time scale such that the particle diffuses over a distance equivalent to several diameters and interacts both colloidally and hydrodynamically with other particles then a long-time diffusion coefficient can be determined[13]. For angular light scattering with a scattering vector, $\mathbf{K} = 10^{-2}$ nm^{-1}, the spatial dimension probed ($2\pi/\mathbf{K}$) is approximately 610 nm in comparison with a particle radius of 85 nm; thus, the time for a particle to diffuse one radius is ~1 ms. As suggested by Pusey and Tough[14] by making measurements at small scattering vectors and long correlation times it is possible to measure long-time self-diffusion coefficients in a particle ensemble. The tracer technique described allows this to be done and in the present work delay times up to 70 ms were utilized.

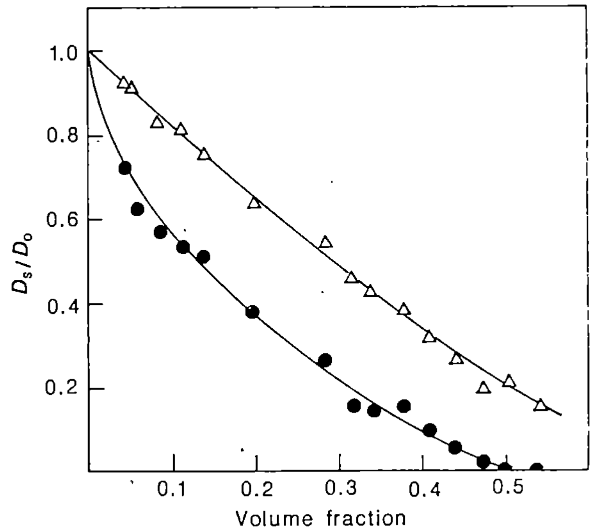

Fig. 1 Ratio of self-diffusion coefficient, D_s, to the single particle diffusion coefficient D_0, as a function of volume fraction: △, short-time self-diffusion; ●, long-time self-diffusion.

The results of the normalized long-time self-diffusion coefficients, D_s/D_0, of the tracer PMMA particles are shown in Fig. 1 as a function of the volume fraction of the dispersion. D_s/D_0 decreases with volume fraction and tends to a very small value as the volume fraction tends towards 0.54. It is of interest to note that Hoover and Ree[15] predicted some time ago that a freezing transition for a hard-sphere system should occur at a volume fraction of 0.495 and melting at 0.545. More recently, Pusey and van Megen[5] reported from visual observations on refractive index matched PMMA dispersions that melting occurs at an effective hard-sphere volume fraction of 0.536. From Fig. 1 it is apparent that the onset of virtually zero diffusional motion is very close to the predicted freezing value of 0.495. It is possible that some motion still appears above this volume fraction but if it does, it is on a very long time-scale. Effectively, the long-term self-diffusional motion, which is movement through the lattice, appears to cease at the freezing transition. The short-time diffusional coefficient, however, which represents the 'rattling' motion of a particle on a lattice site remains finite in this region[9] and as suggested by Pusey[13] is larger than the long-time value.

This is clearly illustrated in Fig. 1 where the results for short-time self-diffusion are also plotted. The theoretical relationship between short-time self-diffusion has been developed theoretically and the results shown in Fig. 1 give good agreement with the multibody treatment of Beenakker and Mazur[16], whereas that for the long-time self-diffusion behaviour is not known beyond the first term in concentration[13] which predicts a less strong dependence on volume fraction. It is to be hoped that this detailed set of experimental results will stimulate further theoretical work in this area.

The results obtained demonstrate the use of monodisperse nearly 'hard-sphere' colloidal particles to simulate physical processes. The tracer technique used in conjunction with photon correlation spectroscopy offers considerable promise as a means of examining dynamic events occurring in concentrated colloidal dispersions.

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Phase changes during elongational flow of polymer solutions

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Protein solutions with a high content of keratin have a tendency to gel on standing. Recent results with synthetic polymers have shown that a shear field can generate the same phenomenon, even in solutions that do not gel at rest. We report here that in a strong elongation flow field, a gel can be induced in polymer solutions that do not gel at rest, or even in a shear field. This throws light on the process of fibre formation by the wet, dry or melt processes, and in addition, gives a possible explanation of the ability of the spider to hang from a freshly extruded thread.

Trouton[1] showed 80 years ago that for newtonian fluids, the elongational viscosity was three times the shear viscosity. However, for non-newtonian fluids, this relationship only applies at the limit of very low deformations. Many polymer solutions show the characteristics of shear thinning (Fig. 1). The elongational behaviour of such solutions is more complicated. Typically, the elongational viscosity will increase, pass through a maximum, and then decrease as strain (or rate of strain) increases. When a polymeric fluid has spinnability[2], the extensional flow may well be characterized by a further increase in elongational viscosity, as the strain asymptotically approaches a limiting value. This allows the fluid to fulfil the conditions for spinnability set out by Lodge[2], that the fluid should get stronger faster than it is getting thinner:

$$\frac{\partial \eta_{el}}{\partial \varepsilon} > \frac{\partial A}{\partial t} \qquad (1)$$

where η_{el} is the elongational viscosity, ε the total (Hencky) strain imposed, A the cross-sectional area, at time t.

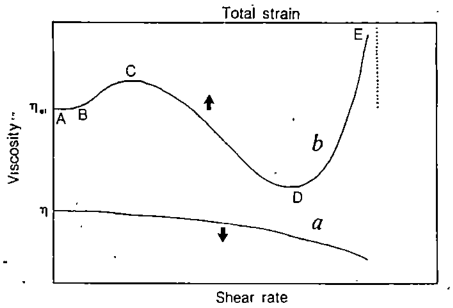

Fig. 1 Typical viscosity curves for a non-newtonian polymer fluid. Curve *a*, shear thinning behaviour, the shear viscosity falling as shear rate increases. Curve *b*, the response in an elongational flow field, divided into distinct regions: AB, Newtonian deformation, $\eta_{el} = 3\eta$; BC, 'strain hardening' deformation of the network structure within the fluid; CD, viscoelastic deformation, with short to medium lifetime entanglement junctions decreasing, causing the fall in η_{el}; DE, elastic deformation of the network held by very long lifetime entanglements (in this region, the fluid is showing solid-like elastic deformation up to a limiting value of strain where cohesive fracture will occur).

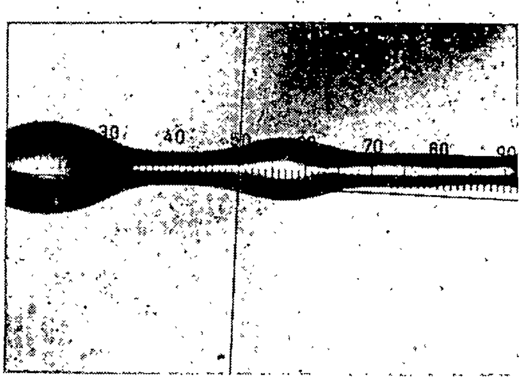

Fig. 2 Filament and droplets of 2% FM9 Copolymer in DPM after elongation had ceased. Viewed under polarized light in a wild M21 microscope system. Each division represents 40 μm.

It had been assumed that this rise in elongational viscosity was due entirely to long lifetime entanglements and increased hydrodynamic volume caused by the uncoiling of macromolecules in the extensional flow field. Recent results[3] show that an additional mechanism can have a part to play and may dominate extensional flow behaviour in certain polymeric systems.

In an experiment rather similar to that described by Trouton, a drop of polymer solution (2% FM9 copolymer in dipropylene glycol monoethyl ether, DPM) was allowed to fall from a burette. The drop fell under gravity, dragging a filament of solution behind it. This filament remained attached to the lip of the burette, and consequently was elongated. The flow was suddenly stopped by the drop hitting a flat surface. The maximum strain imposed was 2.7 units. It was observed that droplets then formed on the filament. These droplets were stable during the timescale of the experiments (up to 6 min). In certain cases, coalescence occurred between two or more droplets. The filament, with attendant droplets, was caught on a modified microscope slide, which held the filament without allowing it to come into contact

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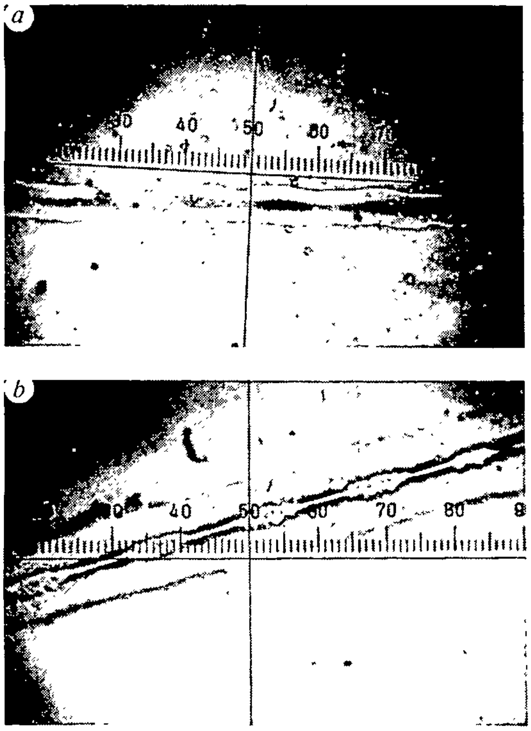

Fig. 3 *a*, Elongated filament of 2% FM9 Copolymer in DPM. Viewed under non-polarized light immediately after being placed on the microscope slide. Each division represents 40 μm. *b*, As *a*, viewed under polarized light, 24 h after being placed on the microscope slide. Each division represents 25 μm.

with the glass surface. The sample was then photographed using a Wild M21 polarizing microscope system. Figure 2 shows a line of high birefringence passing through the centre of an apparently isotropic fluid. The incipient droplets can be seen. In fluid filaments which did not form droplets when elongation ceased, no such line of birefringence could be seen.

In a separate series of experiments, the filament was rapidly extended directly onto the microscope stage, held in extension, and then photographed under non-polarized light. Although there is a slight distortion of the filament, nevertheless, as Fig. 3*a* shows, even with non-polarized transmitted light, a core of gelled polymer could be clearly observed. This indicates, of course, significantly different refractive indices of the gel and of the surrounding fluid. The solvent, DPM, had a boiling point of 188.3 °C at 760-mm pressure, and a partial vapour pressure of 0.36 mm Hg at 25 °C, and so did not evaporate to any great extent. After 24 h, it was observed, Fig. 3*b*, that redissolution of the highly orientated filament was occurring. The solvent-rich phase, being largely isotropic, can be seen surrounding the gelled polymer phase.

These results show conclusively that even in the very short timescale involved in the elongation (<0.6 s), structural changes can be induced even in polymer solutions which are stable at rest. Because these structural changes are reversible, as shown by the redissolution, a weak gelation[4] is observed.

Similar results have been observed with solutions of polymethyl methacrylate (PMMA) in dimethyl methane phosphonate (DMMP), polyethylene oxide (PEO) in water, polyacrylic acid (PAA) in ethylene glycol, polybutadiene (PBD) in dekalin, and others. In all cases, the polymers were of high molecular weight, see Table 1. Where polymers of lower molecular weight ($\leq$50,000) were used, this phenomenon was not observed. The effect was most prominent in those solutions where the solubility parameters of solvent and solute were significantly different; for example, PMMA and DMMP have solubility parameters of 19.1 MPa$^{0.5}$ and 21.6 MPa$^{0.5}$ respectively. In this system, phase change was clearly observed. Therefore, both molecular weight and solvent compatibility are the crucial factors.

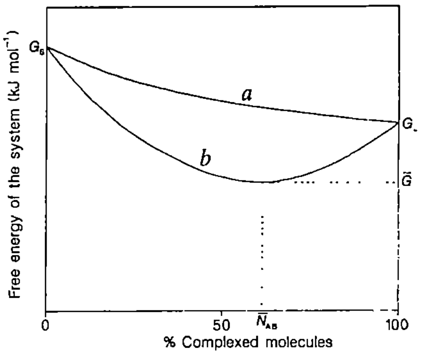

Fig. 4 Free-energy diagram for the complexation process. G_s is the total free energy of the separate constituents in the system. G_c is the total free energy of completely complexed constituents. N_{AB} is the percentage of complexed molecules. At equilibrium, the free energy is given by $\bar{G}$, when $\bar{N}_{AB}$% molecules have complexed.

Gelation can be considered a special case of polymer–polymer complexation. Even in solutions with high intermolecular attraction between similar, or dissimilar, polymers, the formation of complexes is a comparatively long-term process[5]. In extensional flow, an additional factor must be present which is not only speeding up the rate of interaction, but in some cases, is enabling it to occur.

Mackley and Keller[6] have shown that in a converging flow, which is predominantly extensional along the centre line, a very high degree of orientation is induced at high rates of elongation. We have also found[7] that only a comparatively small number of long lifetime entanglements are required to cause this orientation (on average, two-thirds of the molecules have at most one entanglement when the solution changes from having a mainly viscous response to the imposed flow field, to having a mainly elastic response). Therefore, gel formation (complexation) is apparently accelerated in an extensional flow field. We can obtain support for this hypothesis from the thermodynamics of the process.

A typical free-energy diagram for the reversible reaction

$$(\text{polymer}_A) + (\text{polymer}_B) \rightleftharpoons \text{complex} \qquad (2)$$

is shown in Fig. 4. If $G_C > G_S$, then no complexation occurs. If the free energy falls montonically from G_S to G_C, case a, then complete complexation takes place. This happens, for example, with solutions of PAA and poly vinyl pyrrolidone[8]. Another possibility is case b, where the minimum free energy, $\bar{G}$, at equilibrium occurs at some intermediate value of N_{AB}, denoted by $\bar{N}_{AB}$. If $\bar{N}_{AB}$ is small, then very little complexation is taking place. However, if $\bar{G}$ is increased, $\bar{N}_{AB}$ will also increase, until, when $\bar{G} = G_c$, total complexation takes place. The driving force for complexation is given by

$$\Delta G = \bar{G} - G_s \qquad (3)$$

The change in free energy, ΔG, is made up of two components[8],

$$\Delta G = \Delta G_1 + \Delta G_2 \qquad (4)$$

ΔG_1 is the change in conformational free energy, given by

$$\Delta G_1 = \Delta H_1 - T\Delta S_1 \qquad (5)$$

The change in enthalpy of conformation, ΔH_1, is insignificant in comparison to the change in entropy of conformation, ΔS_1.

ΔG_2 is the change in configurational free energy, given by,

$$\Delta G_2 = \Delta H_2 - T\Delta S_2 \qquad (6)$$

ΔH_2 is zero for changes in configuration, whilst ΔS_2, the change in configurational entropy, is related to the probability of the polymers being in solution or in complex[8] and can be quite large.

In a flow field, the free energy of the solution will be increased.

Table 1 Molecular weights of the solutes used in the experiments

Solute	Molecular weight
FM9*	>5,000,000
PMMA	856,000
PEO	4,000,000
PAA	4,000,000
PBD	300,000

* A copolymer of tertiary butyl styrene and a monomer containing 'associating' groups, manufactured by ICI Plc.

In an extensional flow field, the uncoiling and aligning of the macromolecules will decrease ΔS_2 considerably. Hence, the driving force for complexation will increase. If molecules can be held close together by long lifetime entanglements, then complexation will proceed by a 'zipping up' process.

Therefore, it is suggested that the observed rise in elongational viscosity with strain is also due to increased intermolecular interaction between molecules in poor solvents, brought about by alignment in the elongational flow field.

Solutions of silk proteins show an inherent tendency to gel, even at rest[9]. Given high intermolecular forces of attraction an the largely helical conformation of fibroin molecules, it woul seem that on extrusion of an aqueous solution, followed by high elongation rate, the phase change observed in solutions o synthetic polymers would occur. The immediate consequenc of this would be that the complexed gel, being highly orientated would have a much higher tensile strength than a homogeneou solution, enabling the weight of a spider to be supported almos instantaneously. Furthermore, the solvent-rich phase on th outside would be likely to evaporate at a very high rate, increasing the strength of the solidifying filament, although this ha not been observed in the synthetic polymer systems studied.

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^{29}Si magic-angle NMR spectroscopy of low-temperature ordered plagioclase feldspars

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Plagioclase feldspars are among the most abundant silicate minerals in the Earth's crust, and yet their subsolidus phase relations remain, perhaps, the most enigmatic[1]. Their complex behaviour, which involves ordered (commensurate and incommensurate) structures, displacive transitions and three miscibility gaps, is generally attributed to variations in the ordering of aluminium and silicon between tetrahedral sites of the feldspar framework structure as a function of temperature and composition[1,2]. The similarity in X-ray scattering factors of Al and Si atoms, however, has hindered conventional attempts to elucidate the exact structural relations. In the context of feldspar mineralogy, the recent advent of magic-angle sample spinning nuclear magnetic resonance spectroscopy (MAS NMR) has many implications, because it provides a powerful and direct method for determining the local distributions of Al and Si in solids[3–8]. The ^{29}Si-MAS NMR spectra of natural and synthetic plagioclases are equally complex, however, in containing many overlapping peaks and varying according to the chemistry and thermal history of each sample examined[8–11]. We present here new spectra, obtained for a suite of carefully characterized ordered samples from metamorphic and slowly cooled igneous rocks, which shed light on: (1) the sensitivity of the feldspar framework to small amounts of Al/Si disorder; (2) the substitution mechanism of Si for Al in the solid solution from pure anorthite ($CaAl_2Si_2O_8$) towards albite ($NaAlSi_3O_8$); and (3) structural variations in the incommensurate ordered structure of intermediate compositions. In the wider context of silicate chemistry, NMR studies on feldspars provide insights into the subtle influences of continuous solid solution on crystal structures with changing composition. This is also the first attempt to examine the local structure of an incommensurate phase by NMR spectroscopy.

The spectra were obtained for homogeneous, ordered plagioclases which had been purified from natural metamorphic and slowly cooled igneous rocks and had almost all been characterized by transmission electron microscopy, electron microprobe analysis, X-ray powder diffraction and high-temperature solution calorimetry[12,13]. They vary systematically in composition between albite and anorthite which enables us to follow spectral changes across the solid solution (Figs 1, 2). The positions of the best-resolved peaks at ~−111 p.p.m. (parts per 10^6) (T1(0Al)) and −106 p.p.m. (T1(1Al))[11] do not vary significantly (~±1 p.p.m.) across almost the entire compositional range, and our assignments are therefore based on those made for ordered and heat-treated (that is, substantially disordered) Amelia albite[11]. The limited variation with composition of the positions of many of the peaks suggests that the Na/Ca ratio has little direct influence on ^{29}Si-chemical shifts. Peaks in the range −81 to −92 p.p.m. must include those from Q^4 (4Al) sites, as they feature most prominently in the ordered anorthite spectra (Figs 1, 3), whereas those between ~−92 and −112 p.p.m. must be due to Si on Q^4(3Al), Q^4(2Al), Q^4(1Al) and Q^4(0Al) sites, as in ordered and disordered albite.

The spectrum of pure metamorphic anorthite from Val Pasmeda provides the key to behaviour at the anorthite end of the solid solution (Fig. 3*a*). In principle, a fully ordered anorthite (An_{100}) at room temperature should give a spectrum with eight peaks of equal intensity. On heating to ≥240 °C, pure anorthite undergoes a displacive transition, giving a symmetry change from $P\bar{1}$ to $I\bar{1}$ and a reduction in the number of non-equivalent Si-bearing Q^4 (4Al) sites from 8 to 4 (ref. 14). The Val Pasmeda anorthite spectrum has six clearly resolved peaks in three groups, each of two peaks. The intensity ratio of the three groups is 3:3:2, and the spectrum is well simulated by eight peaks with approximately equal intensities if two of the eight have, by chance, the same chemical shift (Table 1, Fig. 3*a*). Because of uncertainties in published refinements of the anorthite structure, however, the assignment of each peak to a specific silicon site cannot yet be made with confidence[9].

Rather than obtain the $I\bar{1}$ structure by heating (not possible at present with our spectrometers), it is possible to investigate the effect of a symmetry increase by following the addition of albite component in solid solution with anorthite, because this effectively depresses the temperature of the $I\bar{1}$ to $P\bar{1}$ transition[15]. If it has local $I\bar{1}$ symmetry, a fully ordered $I\bar{1}$ sample of bytownite should give a spectrum with a maximum of four Q^4(4Al) peaks, and a range of non-Q^4(4Al) peaks which will depend on the

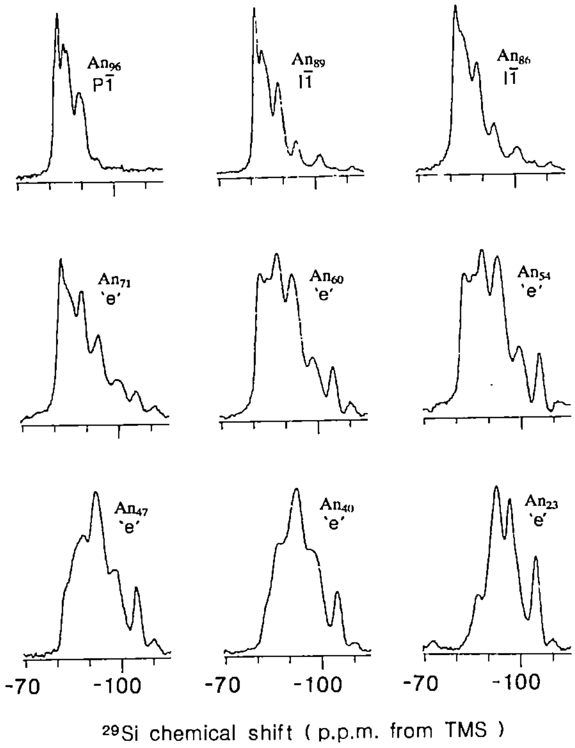

Fig. 1 ^{29}Si-MAS NMR spectra at 8.45 T of a suite of low-temperature, relatively well ordered P$\bar{1}$, I$\bar{1}$ and 'e' plagioclase feldspars. Except for the An$_{71}$ sample, a few grains of which show evidence of exsolution on a fine scale[12], the samples were all free of intergrowth textures[12,13]. The two samples not described previously[12] are 67783a, An$_{46}$ (range An$_{44-48}$), from a granulitic anorthosite from the Uluguru mountains, Tanzania and 84340a, An$_{55}$ (range An$_{51-57}$), from an amphibolite from the Pinnacles, Broken Hill, Australia (sample P6 of ref. 19); both give sharp 'e' reflections in electron diffraction patterns. The spectra were obtained as in ref. 21, except that a sample probe with a very stable rotor assembly was used (Doty Scientific).

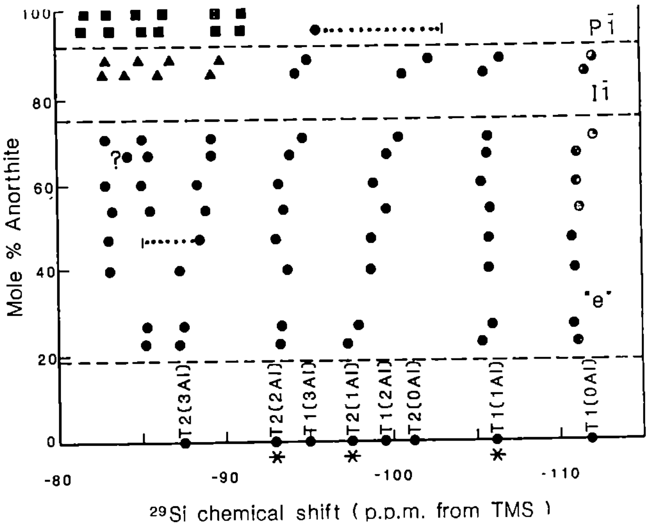

Fig. 2 Variation with bulk composition of the positions of the readily resolvable peaks and shoulders in the ^{29}Si-MAS NMR spectra of our plagioclase samples. ■, Q^4(4Al) peaks of P$\bar{1}$ samples, ▲, Q^4(4Al) peaks of I$\bar{1}$ samples; ●, other peaks; ... I, broad band of intensity; peak assignments for pure albite are for low albite (indicated by *) and disordered albite[11]. Note the lack of variation in the positions of the peaks for the T1(0Al) (−111 p.p.m.) and T1(1Al) (−106 p.p.m.) sites. The positions of the peak maxima indicate that in the −97 to −102 p.p.m. range, peaks for T2(1Al) sites dominate the spectra of low An-content samples, and peaks for T1(2Al) and T2(0Al) sites become relatively more intense with increasing An-content; in the −92 to −95 p.p.m. range, peaks for T2(2Al) sites dominate at low and intermediate An contents, and the peaks for T1(3Al) sites become relatively more intense at higher An contents. In the −87 to −91 p.p.m. range, peaks for T2(3Al) sites dominate at low An contents and the peaks for Q^4(4Al) sites become more intense with increasing An content. Precision and accuracy of the peak positions is ~±0.5–0.8 p.p.m.

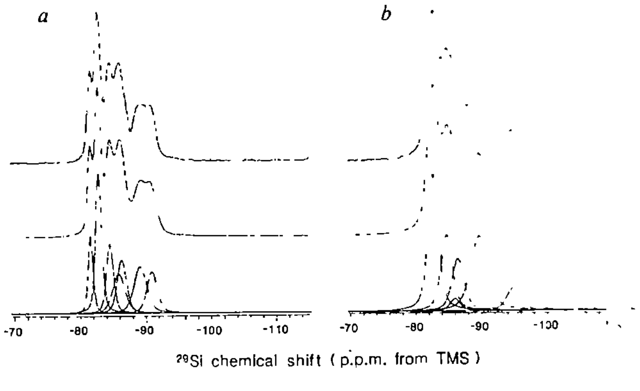

Fig. 3 *a*, Observed and simulated spectra and spectral components for the 8.45-T ^{29}Si-MAS NMR spectrum of Val Pasmeda metamorphic anorthite (An$_{100}$); simulation obtained with the Nicolet curve fitting program CAP. *b*, Observed and simulated spectra and spectral components for the 8.45-T ^{29}Si-MAS NMR spectrum of the metamorphic Sittampundi bytownite (An$_{89}$); simulation obtained with the Nicolet curve fitting program CAP.

sites occupied by the additional Si substituting for Al. The spectrum of metamorphic bytownite (An$_{89}$) in Fig. 3*b* shows just such a decrease in the number of peaks in the range −82 to −90 p.p.m. and has several additional peaks in the range −90 to −112 p.p.m. This interpretation is contradicted, however, by the presence of diffuse reflections at $h+k=2n$, $l=2n+1$ positions in electron diffraction patterns from the sample[12], and by the observation that the relative intensities of the Q^4(4Al) peaks in the NMR spectrum are not approximately equal; the local-symmetry within the crystals (over the range of a few unit cells) may really be P$\bar{1}$. The NMR and diffraction observations can be reconciled if the positions of the Na and Ca atoms do not directly influence the ^{29}Si-chemical shifts, but are primarily responsible for the diffuse reflections. Alternatively, the feldspar framework is not distorted sufficiently from I$\bar{1}$ symmetry to give resolvable peak splitting in the NMR spectrum, despite the effects of small P$\bar{1}$ displacements of all the atoms. In this context, note that the spectra of synthetic and volcanic anorthites show significant peak broadening and overlap effects[9,10] relative to metamorphic anorthite, even though they may have only a small amount of Al/Si disorder[12]. The ^{29}Si-spectra thus provide information relating not only to different local tetrahedral site occupancies but also to the nature of framework distortions. There seems to be a rather sensitive relationship between these distortions and the degree of order.

Using the assignments for high albite of Yang *et al.*[11], non-Q^4(4Al) peaks in the An$_{89}$ sample have been assigned primarily to T1(0Al) (−112 p.p.m.), T1(1Al) (−106 p.p.m.), T2(0Al) (−102 p.p.m.) and T1(3Al) (−95 p.p.m.); signal from other sites may be lost in the larger peaks. A first attempt at a simulation of the spectrum has been made including all these peaks and contributions from T2(3Al) (−88 p.p.m.), T1(2Al) (−100 p.p.m.), T2(1Al) (−97 p.p.m.) and T2(2Al) (−92 p.p.m.) sites (Table 1, Fig. 3*b*). Because of the strict alternation of Si and Al sites in fully ordered anorthite, each additional Si atom replacing an Al atom forms a Q^4(0Al) site and causes the neighbouring sites, of which there are four, to become Q^4(3Al).

Table 1 Peak positions, relative peak intensities, and peak assignments for the simulated ^{29}Si spectra of Val Pasmeda An$_{100}$ and Sittampundi An$_{89}$

Peak position	Relative intensity (%)	Site assignments
Val Pasmeda An$_{100}$		
−81.7	11.5	Q^4(4Al)
−83.0	25.1	Q^4(4Al)
−84.8	11.9	Q^4(4Al)
−85.5	12.7	Q^4(4Al)
−86.3	12.4	Q^4(4Al)
−89.6	13.6	Q^4(4Al)
−91.1	12.8	Q^4(4Al)
Sittampundi An$_{89}$		
−82.8	29.0	Q^4(4Al)
−84.9	15.8	Q^4(4Al)
−86.3	13.8	Q^4(4Al)
−88.4	3.9	T2(3Al)
−89.7	23.7	Q^4(4Al)
−91.7	0.54	T2(2Al)
−94.9	7.6	T1(3Al)
−96.5	0.18	T2(1Al)
−100.5	0.29	T1(2Al)
−102.2	4.1	T2(0Al)
−106.5	0.55	T1(1Al)
−112.0	0.67	T1(0Al)

An$_{100}$ simulated assuming P$\bar{1}$ local structure (eight Si sites); An$_{89}$ simulated assuming I$\bar{1}$ local structure (four Si sites).

If such a substitution mechanism extends to An$_{89}$, 5.2% of the Si sites should be Q^4(0Al), which compares quite closely with the total intensity (4.8%) of the two peaks assigned to this type of site in the simulation. The apparent preponderance of T1(3Al) sites (−95 p.p.m.) relative to T2(3Al) (−88 p.p.m.) and of T2(0Al) (−102 p.p.m.) relative to T1(0Al) (−112 p.p.m.) implies also that the excess Si substitutes preferentially for Al on T2 sites rather than on T1 sites. (Each T2 site in the feldspar framework shares oxygens with three T1 sites and one T2 site, whereas each T1 site shares oxygens with three T2 sites and one T1 site[14].) Recent structure refinements of bytownites are consistent with this preference of Si for T2 over T1 sites[16,17]. If the excess Si prefers T2 to T1 by five to one but is otherwise randomly distributed, the expected concentration of T1(1Al) sites (at −106 p.p.m.) is 0.2%, compared with the observed concentration of 0.55% in the simulation. This observation is consistent with some clustering of the sites occupied by the excess Si.

The most striking feature of the spectra of the plagioclase crystals with incommensurate (or 'e') ordered structures is the narrowness of their most prominent peaks (Fig. 1). These spectra must be made up of a number of overlapping peaks, but the presence of some relatively intense and narrow peaks implies that there are many Si sites in the crystals with identical environments. Presumably, therefore, the incommensurate structure possesses a rather high degree of Al/Si order. Certainly, none of the spectra we have seen for disordered plagioclases of any composition has such well-resolved peaks. Other experimental studies have suggested that there may be significant differences between 'e' structures in the composition ranges An$_{50}$–An$_{70}$ (designated 'e$_1$') and An$_{20}$–An$_{50}$ (designated 'e$_2$')[1,2,12,13,18,19]. The MAS NMR spectra appear to reflect these differences (Figs 1, 2). Spectra from the 'e$_1$' samples (An$_{54}$, An$_{60}$, An$_{71}$) all contain well resolved peaks or shoulders at ~−83, −85 and −89 p.p.m., presumably due to Q^4(4Al) sites of similar character to those in An$_{89}$. With increasing Ab content in this composition range, the relative intensities of the Q^4(4Al) sites decrease (except for the peak at −89 p.p.m., which overlaps with the T2(3Al) peak) and the total relative intensity of the peaks for non-Q^4(4Al) sites increases. The non-Q^4(4Al) sites present, however, are not entirely those of either ordered low albite or disordered high albite. In particular, there is little intensity for T2(1Al) sites at ~−97 p.p.m., a dominant peak in albite spectra[9-11,20-22]. The spectra of 'e$_2$' plagioclases with compositions from An$_{23}$ to An$_{40}$ (and possibly the An$_{47}$ sample) contain significant intensity for the three peaks of low albite at −93, −97 and −105 p.p.m. but do not contain well-defined peaks for Q^4(4Al) sites. These spectra will provide a basis for testing site-occupancy models of the incommensurate plagioclase structures, but are not easy to interpret directly. For the present, they merely support the view that slab models involving only layers of albite and anorthite cannot be correct[1,9,12,13,23,24], that the incommensurate structure really is rather well ordered, and that the distinction between 'e$_1$' and 'e$_2$' incommensurate types may be structurally significant.

We conclude that the ^{29}Si-MAS NMR spectra for plagioclase feldspars contain a wealth of quantitative structural information. We are attempting to unravel some of the details by systematic studies of specially selected and characterized samples and by simulation of the spectra with different structural models of 'e' plagioclases. Distortions of the feldspar framework are particularly sensitive to relatively small variations in Al/Si order and the NMR technique emphasizes this sensitivity. In contrast, we suggest that the peak positions are relatively insensitive to variations in Na/Ca content. This type of approach to silicate chemistry gives insights into details both of the structures of individual stoichiometric phases and of the local structure variations within crystals which are due to the formation of continuous solid solutions.

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Structure of the Kenya rift from seismic refraction

KRISP working group*

The Kenya rift forms part of the East African rift system, a tensional feature sometimes regarded as representing an early stage of continental break-up[1,2]. High-resolution seismic refraction data are required to provide constraints on models of the deep structure and processes. A 300-km seismic refraction line along the axis of the southern portion of the rift shows variations of up to 4 km in the depth to the basement. A 7.6 km s^{-1} layer at ~35 km depth beneath the rift is overlain by a 10-km-thick lens which appears to include anomalous low-velocity material. This lens is interpreted to represent the base of the crust and thickens beneath the Kenya dome. There is no clear evidence for a wide high-velocity intrusive zone in the upper crust within the rift.

A long-range explosion seismology experiment was carried out in August 1985 as part of KRISP 85 (Kenya Rift International Seismic Project 85) by a group of institutes from the United Kingdom, United States, West Germany, Switzerland, Kenya and Zimbabwe. The major aims of this experiment were (1) to determine the velocity-depth structure beneath this portion of the rift and (2) to test shot efficiency, recording station characteristics, and transmission along and across the rift and its boundaries. This information is required for the planning of a series of further experiments in 1988 and beyond.

The experiment consisted of two seismic refraction lines (Fig. 1). Along the rift, a N-S line was completed with shots at Lake Baringo, (BAR), Chepkererat (CHE), Solai (SOL), Elmenteita (ELM), Naivasha (NAI), Susua (SUS), and Magadi (MAG) recorded by 42 three-component stations at 3.5 km intervals between CHE and SUS. Across the rift an E-W line was completed with shots at Ewaso Ngiro (EWA), Ntulelei (NTU), Susua (SUS), Mount Margaret (MAR), and Makuyu (MAK) into the same stations deployed at 1.25 km intervals between NTU and MAR. On both lines, 8 additional stations were used to fill gaps between the end and off-end shots at larger intervals. The explosion programme was followed by three earthquake recording experiments between September and December 1985.

In the vicinity of the seismic experiment, the rift is 50–70 km wide (Fig. 1) and is flanked by shoulders 1–2 km above the rift floor, the major boundary faults having throws of up to 3–4 km. It is covered by volcanics and sediments[2-4] to a depth of ≥2 km and is dissected by subparallel, north-south trending normal faults. Precambrian rocks are exposed in rift boundary faults to the north (Elgeyo escarpment) and south (Nguruman escarpment) of the central section, and in the Saimo fault block within the rift to the east of the Elgeyo. The oldest rift structures are mid-Tertiary and immediately pre-date the rift volcanism[3] which began in the Miocene about 23 Myr ago[2] and has continued to the present as is evident by the abundant volcanic craters (Fig. 1).

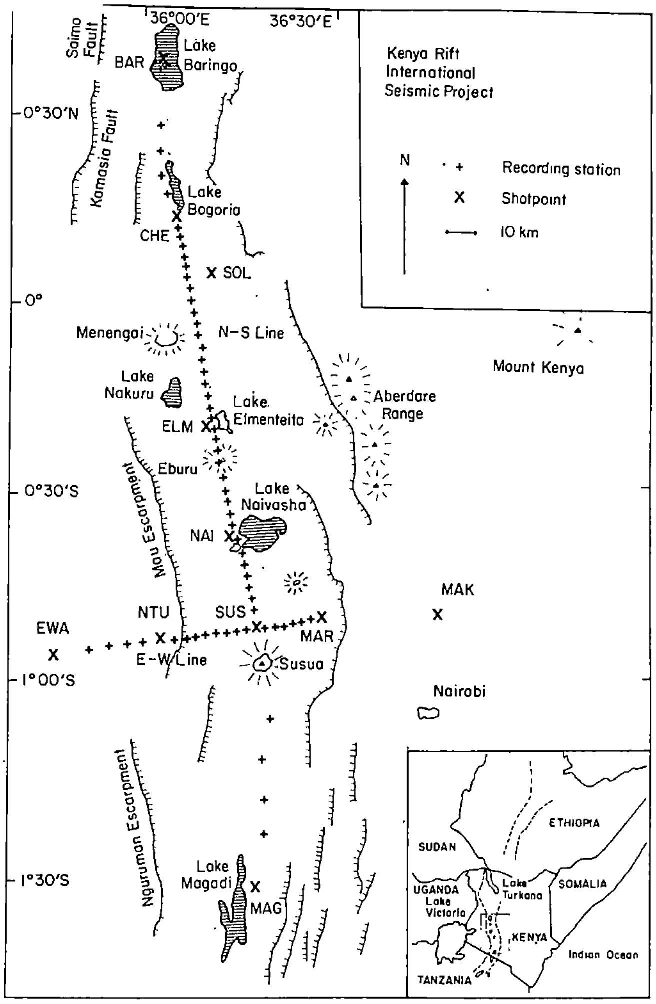

Fig. 1 Location of the KRISP 85 seismic experiment showing the lines and shot points. Recording station locations for the east-west line are diagrammatic, as the spacing is too small to be shown on this scale.

Geophyscial models based on gravity[3,5,6] and teleseismic data[7] suggest first, lithospheric attentuation, enabling anomalously low-velocity, low-density asthenospheric material to reach to the base of the crust beneath the Rift Valley and thus replace a wide mantle zone. It is suggested as a mechanism providing isostatic compensation for the raised topography of the Kenya dome[8]; and second, the models suggest the presence of a relatively high-velocity, high-density intrusive body penetrating the crust to shallow levels from the anomalous mantle zone beneath the axis of the rift.

The location of our north-south line was chosen to overlie this 'axial intrusion' as closely as possible. A seismic refraction line completed in 1968 along the rift axis to the north of Lake Bogoria[9,10] also suggests asthenospheric penetration of the lithosphere to within 15–20 km of the surface. A shorter refraction line completed in 1975 west of Lake Baringo[4] failed to give any direct evidence for the existence of the axial intrusion, although the presence of anomalously high density material in the upper crust was implied by the combined interpretation of the refraction and the gravity data.

Recording stations were positioned by conventional and aerial survey techniques to an accuracy of 20 m. Just under half of the data were recorded digitally on US equipment (Kinemetrics

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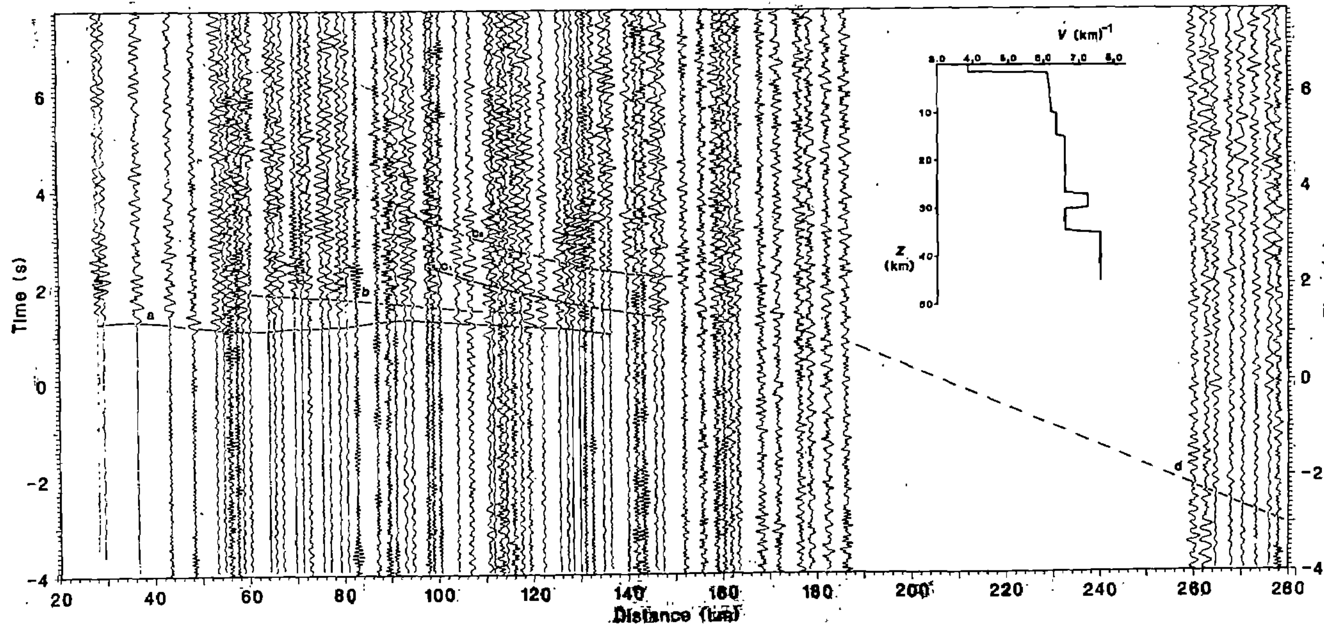

Fig. 2 Record section from shots BA1 and BA2 with phase correlations and velocity-depth function. Phase d is continued across the data gap for the reader's convenience. (Reducing velocity = 6.0 km s^{-1}).

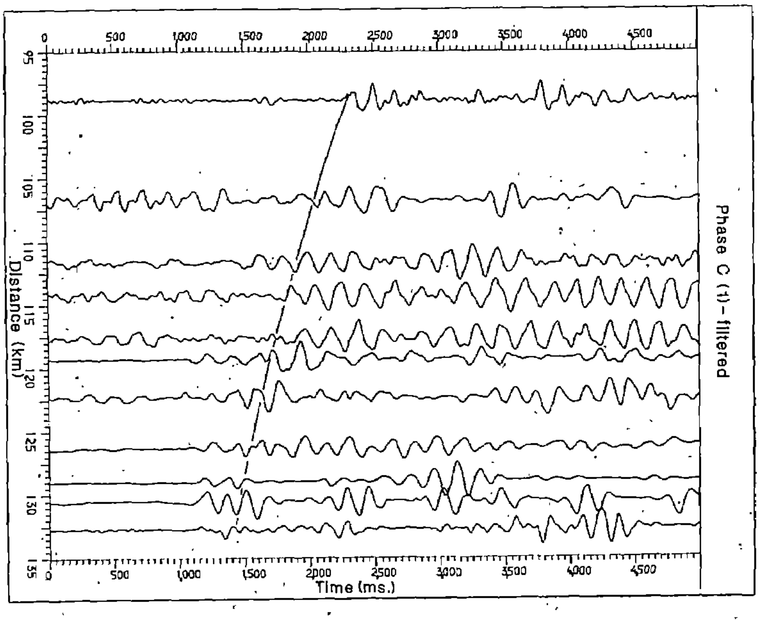

Fig. 3 Enhancement of phase c_1 by polarization filtering (reducing velocity = 6.0 km s^{-1}).

DR100 and University of Wisconsin recorders) and the rest on frequency-modulated analogue systems (European MARS and UK Geostores). At each station an Omega-controlled time signal was recorded in addition to the three seismic components. The analogue data were subsequently digitized and merged with the digitally recorded data at 100 samples per second.

The lake shots were efficient. For example, as can be seen on the section shown on Fig. 2, a 1-tonne shot in Lake Baringo provided useful energy to 280 km. In contrast, the borehole shots were inefficient—a 1-tonne shot distributed between six holes at 60 m depth at MAG did not provide useful energy beyond 100 km. Two factors contributed to this—(1) the shot holes did not reach the water table and (2) the recording stations were largely on pyroclastics which were associated with high noise levels. This was particularly serious on the east-west line as no lakes were available. For this reason, the data for this line are not included in the analysis at this time. However, this experience of borehole shooting in Kenya has provided the information for the planning of future experiments which was one of the objects of the programme.

Frequency filtering, and 'REMODE'[11]-type polarization filtering techniques were applied to the data to improve the signal to noise ratio. A P-wave record section of the data for the two shots at Baringo is shown in Fig. 2 together with our subjective phase correlations. The traces shown have been bandpass-filtered between 1 and 20 Hz and normalized. Figure 3 shows a portion of the same data after the application of a polarization filter. Fuller presentations of the data will be made elsewhere[19].

Analysis of phases observed up to a few tens of kilometres from shots on our north-south line and previous experiments provides evidence that the rift infill has velocities ranging from 1.4 to 4.6 km s^{-1} and thicknesses ranging from 2 km beneath BAR and SUS to 6 km beneath NAI (Fig. 4). Phase a (P_g) can be recognized as a first arrival on the record section up to about 130 km with an apparent velocity of about 6.0 km s^{-1}. From the reverse shot, NAI, phase a has a higher apparent velocity and

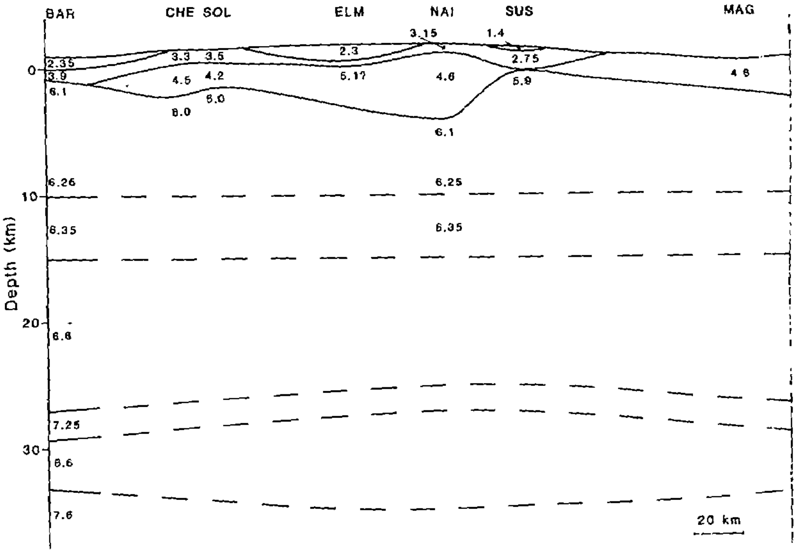

Fig. 4 Proposed velocity-depth structure along the axis of the rift from Lake Baringo to Lake Magadi (velocities shown in km s^{-1}).

thus the two-dimensional model shows the uppermost basement dipping southwards between Elmenteita and Naivasha with velocities of about 6.1 km s^{-1} increasing to 6.25 km s^{-1} at 10 km depth. Phase b can be observed with high amplitudes between distances of 70 and 120 km. It is interpreted as a reflection from an intercrustal discontinuity at about 15 km depth. Between 15 and 27 km the velocity is about 6.6 km s^{-1}. Phase c_1 can be recognized, especially on the polarization filtered record section (Fig. 3) produced from selected three-component stations at distances between 100 and 130 km. It is interpreted as a reflected phase from a discontinuity at about 27 km depth below which a velocity of about 7.25 km s^{-1} occurs. Phase c_2, interpreted as a reflection from a discontinuity at about 35 km depth, can be observed beyond about 100 km. In order to produce the large amplitudes for this phase at distances around 100 km, a layer with a low velocity of about 6.6 km s^{-1} is required between 30 and 35 km depth. On the record section, a few first arrivals occur in the 260–280 km range at −2 to −3 seconds reduced time. Because of their high amplitudes these arrivals are interpreted as diving waves (phase d) within a layer of velocity 7.6 km s^{-1} at its top. The main features of the velocity–depth function are shown in Fig. 2. Travel-time and amplitude analysis of the phases in the 50–150 km range suggest that the velocities shown are maximum estimates. Decreases of 0.1 and 0.25 km s^{-1} in the 6.6 and 7.25 km s^{-1} would provide acceptable fits to the data. However, these decreases will affect the depth estimates to the 7.25 and 7.6 km s^{-1} layers by less than 2 km.

The traditional definition of the Moho is not easily applied to this area of unusual velocity structure. The widely accepted definition is "that level in the earth where the compressional wave velocity increases rapidly or discontinuously to a value between 7.6 and 8.6 km s^{-1}" (ref. 12). However, from deep seismic sounding experiments in Afar and Ethiopia, Berckhemer *et al.*[13] and Ruegg[14] attribute velocities as low as 6.8 km s^{-1} to the pper mantle. Regardless of whether we call the 7.2 or 7.6 km s^{-1} ayer the uppermost mantle, if we assume that the interpretation f the 1968 refraction data[9] is correct, then our results demontrate a thickening of the crust along the rift axis towards the entre of the Kenya dome. This thickening is consistent with he regional gravity data[15,16] which shows that gravity values ecrease from greater than −100 mgal around southern Lake urkana through about −160 mgal near Lake Baringo to about 200 mgal near Lake Naivasha.

Banks and Swain[8] studied the isostatic response of an 800 km uare area surrounding the Kenya rift and favoured the idea at the compensation of the Kenya topographic dome is cause of anomalous, low density material in the upper mantle. owever, the results presented here suggest the compensation is due, at least in part, to crustal thickening.

It has long been suggested that an 'axial intrusion' rising to very high levels in the crust is responsible for the gravity high along the axis of the rift[3,5]. The basement velocities (6.1 km s^{-1}) measured by our experiment are rather low to be associated with such a dense intrusion so it is natural to ask if some other feature could account for the gravity high. Fairhead[17] suggested a combination of volcanic infill and a relatively narrow dyke to model a profile along our east–west line. His interpretation mainly differed from Searle's[5] in its choice of regional field which (unlike Searle's) was assumed to follow closely the observed Bouguer anomaly over exposed sections of the basement both west and east of the rift. This regional field yields positive residuals along the rift axis (+20 to 30 mgal) which cannot therefore be due to sub-volcanic basement topography within the rift. The source must lie within the basement. The 6.1 km s^{-1} could represent intrusive material, since lower basement velocities have been measured off the axis[4], and velocity increases with depth (possibly indicative of dyke injection).

Savage and Long[7] suggest that the short-wavelength pattern of teleseismic P-wave delays between Kaptagat and Nairobi is also primarily due to the existence of the high-velocity, axial intrusion rising to a level of 10–20 km within the rift. Since our results do not support the existence of such a feature continuously along the line, we suggest that variations in crustal structure (in particular basement topography) both across and along the rift axis make an important contribution to the reported pattern of P-wave delays. Further work on the proposed axial intrusion is clearly necessary as it has an important bearing in the low angle detachment models now gaining favour[18].

In Fig. 4 we present a preliminary model synthesizing the results of the KRISP 85 experiment and relevant data from previous studies. The model demonstrates that there is considerable basement topographic relief. The most important new feature of the velocity–depth model is the presence of a 10-km-thick lens at a depth of 25 km of material with a velocity of 7.2 km s^{-1} at its top decreasing by about 10% at the base. The study also suggests that there are significant variations in the crustal structure along the axis of the rift. Both of these features require further investigation.

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Late-Holocene upper timberline variation in the southern Sierra Nevada

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Alpine timberline has been shown to be a definite tension zone between trees and climate where trees invade higher ground during favourable climatic periods and retreat during periods of deteriorating conditions[1]. These movements are indicative of an upper forest limit sensitively adjusted to climatic variation. Here I present absolutely dated dendrochronological records from living trees and standing snags and radiometric dates from relict logs of foxtail pine at Cirque Peak, southern Sierra Nevada, California, providing the first detailed record of temperature-induced mid- to late-Holocene fluctuations in Sierran timberline. Marked declines in timberline correspond to lichen-dated cold periods and/or glacial advance.

Chronologies of variation in altitude of upper timberline reflecting late-Holocene climatic change have been established for several alpine areas in western North America[2–6]. These timberlines are characterized by zones of standing snags at and just above present timberline, rooted and fallen snags above this zone, and small deeply weathered remnants at the highest limit of relict wood. Similar conditions exist in several areas of the Sierra Nevada, with relict timberlines being especially well preserved in the drier southern portion of the range.

Foxtail pine (*P. balfouriana* Grev. and Balf.) is found at the timberline on Cirque Peak in the southern Sierra Nevada (Fig. 1). This species is closely related to the Great Basin bristlecone pine (*P. longaeva* Bailey) found 60 km northeast in White and Inyo Mountains. Foxtail pine is characterizec high temperature sensitivity at its upper growth limit, excel cross-dating qualities, and a relatively long lifespan. Living relict individuals with lifespans exceeding 1,200 yr have t found at Cirque Peak[7].

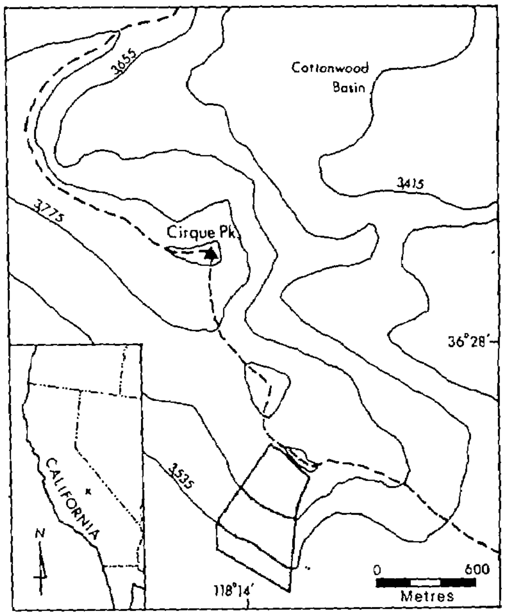

Fig. 1 Topographic map showing the Cirque Peak upper timbe line site (shaded) and its relationship to Cottonwood Basin. Elev: tions in metres.

Computer-aided cross-dating[8] of relict wood above the p ent timberline permitted the construction of an absolutely d: 3,031-yr chronology of ring-width variation for the Cirque I site[7]. Samples from 6,300–3,100 yr BP were dated radiometric using the earliest portion of the remnant. Sample elevation recorded at the time of collection and used in conjunction dendrochronological and calibrated[9] radiometric sample to produce a chronology variation in timberline eleva (Fig. 2).

Timberline was defined as the upper limit at which trees continually established, a position now indicated by a sl transition from upright trees to isolated outlier krumm forms. The minimum timberline position for each time pe was inferred from five to six cross-dated samples of upr non-krummholz wood.

The oldest and highest sample located is a small weath remnant 68 m above the present timberline, radiocarbon da at 6,300±300 yr BP (UCLA 2418-A). The wide growth ring this and other cross-dated remnants at this elevation are inc tive of warm, highly favourable growing conditions during Altithermal. A date[9] of 3,530±50 yr BP (UCLA 2463-C) sample 65 m above the present timberline indicates maximum levels were maintained until at least 3,500 yr BP

The possibility of a higher Altithermal timberline, simil that documented in the White Mountains[2,3], was explore length. Slopes with suitable environmental factors, and lac physical characteristics that would inhibit upward movemer the timberline, extend nearly to the crest of the range (24 above the present timberline). But so far no relict wood been found higher than 70 m above present levels for up to 1 from the study site. In the light of the observed excellent sam preservation and lack of fire scars it appears unlikely that timberline at Cirque Peak was much higher than 70 m abov present level at any time since 6,300 yr BP. Relict timberline other local peaks suggest that this may represent the maxim Altithermal timberline elevation for the region.

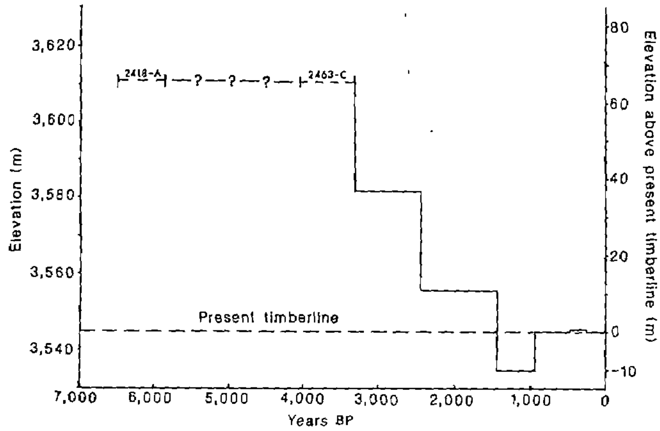

Fig. 2 Elevation of mid- to late-Holocene timberline at Cirque Peak. Sample numbers of UCLA radiocarbon dates are shown at the highest level of remnant wood 65-68 m above the present timberline.

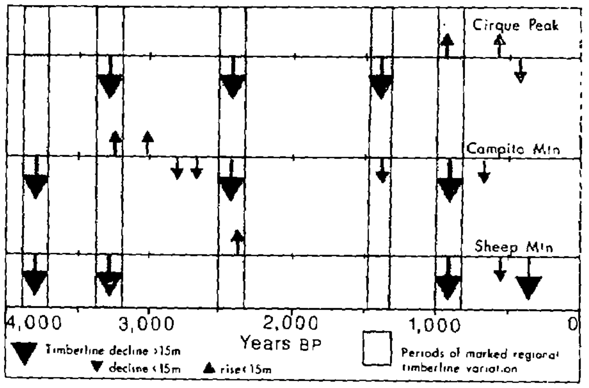

Fig. 3 Major timberline elevation changes at Cirque Peak and neighbouring White Mountain sites. Periods of synchronous timberline variation are indicated at approximately 3,800, 3,400, 2,400, 1,400 and 900 y BP, indicating possible regional climatic forcing of the upper timberline position.

High Altithermal levels were followed by a rapid decline in timberline of 30 m between 3,400 and 3,200 yr BP. Extrapolation of sample ages to pith dates shows that a large number of trees were established 30-37 m above present levels between 3,200 and 2,800 yr BP. This suggests that the timberline stabilized ~35 m above present levels by 3,200 yr BP and possibly several hundred years earlier.

Many trees, especially those at elevations greater than 20 m above the present timberline, died between 2,500 and 2,300 yr BP. A marked decrease in ring widths in all samples at 2,400 yr BP is suggestive of colder conditions leading to decreased growth and regenerative potential. A study of late-Holocene glaciation in the adjacent Cottonwood Basin[7] indicates that this period was one of marked glacial advance, correlating to the earliest Recess Peak glaciation in the Sierra Nevada[10]. The timberline fell ~25 m to 12 m above the present timberline at this time, where it stayed for ~900 yr. Between 1,400 and 1,300 yr BP the timberline fell rapidly to 10 m below its present level. This decrease coincides with a sharp decline in ring widths for all trees from 1,400 to 1,000 yr BP. Similar small ring widths are also found between 1,300 and 1,050 yr BP at Sheep Mountain in the White Mountains[11]. Lichenometric dating of deposits in the adjacent Cottonwood Basin[7] and the Mammoth Lakes region further north[10] indicates possible increased glacial activity during this interval.

The period 950-850 yr BP was one of increasing tree establishment during which the timberline rose 10 m to its present level. A few trees were established marginally above present levels at 450 yr BP, suggesting that the timberline may have peaked at this time. The onset of Matthes 'little ice age' advances at 350 yr BP does not appear to have significantly affected timberline position. The establishment of some small erect seedlings 5-10 m above the present timberline since 100 yr BP suggests a renewed rise in timberline due to recent warmer conditions[2,3] r possible increases in atmospheric carbon dioxide concentraion[12]. It remains to be seen if these trees will survive to maturity r if they represent short-term variations in timberline that are ot resolvable in the context of the coarser record of timberline.

Comparison with timberline records from the White and ocky Mountains shows several points of similarity as well as me divergence between the records. Of note is the net ifference in timberline elevation between present and past vels, especially from 6,300 to 3,500 yr BP. The Sierra Nevada nd White Mountain timberlines were both at maximum elevaon during this period, but the Cirque Peak timberline was only m above its present level whereas the Campito and Sheep ountain timberlines were 110 and 150 m respectively above heir present levels[3]. Pollen evidence of coarser resolution from the Rocky Mountains indicates that timberline was then 70-100 m above present levels.

The regular nature of the current Cirque Peak timberline, which is characterized by a sharp transition from upright trees to treeless conditions and a smooth drop in elevation from southwest to northwest slopes, suggests that this timberline is a true 'climatic' timberline. The upper limit of remnant wood parallels the current timberline and also shows a similar distinct boundary. The sharply defined timberline and apparent lack of site specific microclimatic and lithological forcing of timberline position is in marked contrast to other more variable upper timberline limits in western North America. This regularity, as well as differences in defining timberline position, may account for a portion of the observed net elevation difference between localities.

A timberline 10 m below present at Cirque Peak between 1,400 and 950 yr BP may be a reflection of the shorter average lifespan of foxtail pine than bristlecone pine. This difference in longevity makes foxtail pine less likely to survive unfavourable periods, and thus more sensitive (ring width) and responsive (elevation change) to climatic variation than longer-lived species. Thus, the Cirque Peak record for this period may indicate cold conditions and a related glacial advance not readily apparent in other upper timberline records.

Mid- to late-Holocene variations of Cirque Peak timberline mirror the timing of major changes in timberline elevation in the White Mountains[3] and, with the exception of the rise to current levels at 950 yr BP, the direction of specific timberline changes at Campito Mountian (Fig. 3). A similar decreasing trend in timberline elevation similar to that at Cirque Peak has been observed in the Rocky Mountains[6]. This correspondence supports a view of regional climatic forcing of timberline elevation. An additional correspondence of timberline decline with glacial advance in the Cirque Peak region[7] suggests that the chronology of the Cirque Peak timberline can provide a link to the evaluation of climatic conditions leading to glacial advance and retreat in the Sierra Nevada and possibly the western United States.

Long, continuous and precisely dated records of timberline variation are uncommon and are especially valuable in the Sierra Nevada where, due to lack of absolutely datable material, little radiometric dating of climatically induced events such as glaciation has been possible. The Cirque Peak timberline and associated annual ring-width variation record, in conjunction with pollen and relative-age-dated deposits, will provide additional information about the physical environment in the Sierra Nevada over the past 6,300 yr. The integration of this record with those from the White Mountains will help isolate the principal mechanisms responsible for climatic fluctuations during the mid to late Holocene and allow the evaluation of climatic spatial variability.

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Decrease in precipitation acidity resulting from decreased SO_4^{2-} concentration

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The effect of decreases in SO_2 emissions on precipitation acidity has received much attention[1-10], but there has been no direct quantification of how recent decreases in SO_2 emissions in the northeastern and midwestern United States have affected precipitation acidity at a local site. Yet such quantification is an important step in assessing the effectiveness of control measures for SO_2 emissions. It is thought that recent decreases in SO_4^{2-} concentration in precipitation at the Hubbard Brook Experimental Forest (HBEF), New Hampshire, USA, result from decreases in SO_2 emissions. The effect of the SO_4^{2-} decrease on precipitation acidity is obscured by long-term trends in other ions, which also influence acidity. Here we show that, given the observed trends in concentration of other ions, the H^+ concentration in 1983 would have been nearly two-thirds higher than the measured values if the SO_4^{2-} concentration had not decreased.

During the 20-year period 1964-1983, decreases in concentration and deposition of SO_4^{2-} (Fig. 1*a*) in precipitation at HBEF have been attributed to decreases in SO_2 emissions in the northeastern and midwestern USA (Environmental Protection Agency regions I, II and V)[10,11]. Significant decreasing trends in SO_4^{2-} concentrations have also been shown at 5 out of 8 National Atmospheric Deposition Program monitoring sites in the northeastern and midwestern USA since their inception in 1978[12]. There is also evidence of a close relationship between SO_2 emission and SO_4^{2-} deposition from the western USA[7], and indications of a similar relationship in Europe[13].

Air masses from the Atlantic Ocean contribute <3% to the total SO_4^{2-} deposition at HBEF[14]. Locally derived soil dust is a small component[15] and unlikely to contribute significant amounts of SO_4^{2-}. Atmospheric sulphur in the northeastern USA derives almost entirely from anthropogenic SO_2 emissions: this is further evidence that the decreases in SO_4^{2-} (Fig. 1*a*) at HBEF are due to decreases in emissions of SO_2 (refs 8-10, 16-19).

Here we quantify the effect of these decreases in SO_4^{2-} on precipitation acidity, using precipitation chemistry from bulk precipitation monitors during a 20-year period at HBEF. emphasize, however, that although we assume that the decre in SO_4^{2-} (Fig. 1*a*) at HBEF are a result of decreases in emissions, our calculations are not dependent on an underst ing of the specific shape of the relationship between emission and SO_4^{2-} deposition, nor on a knowledge of the rela contribution of local versus distant emission sources. Our sa ling and analytical methods have been described elsewhe

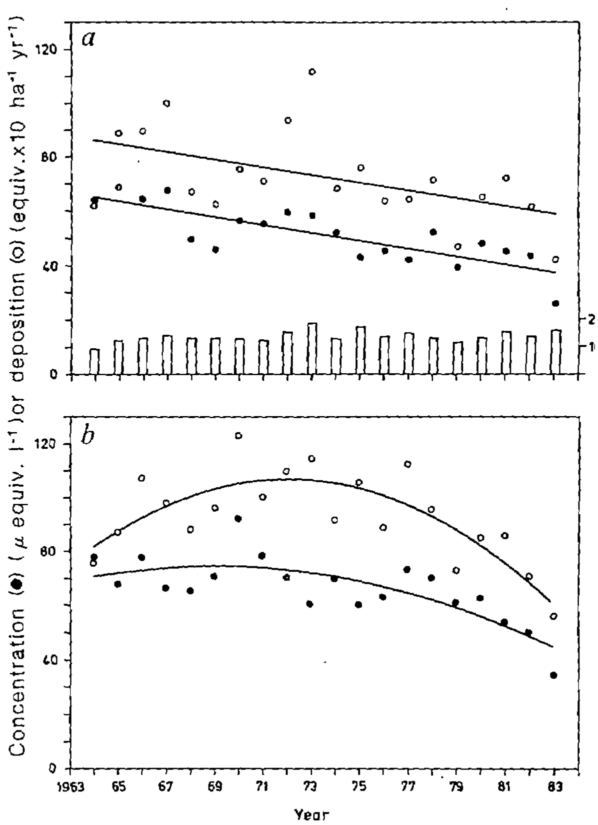

Fig. 1 Annual volume-weighted average ion concentration (and deposition (○) for SO_4^{2-} (*a*) and H^+ (*b*) in precipitation watershed 6 of HBEF for 1964-83. Lines represent significa ($P \leq 0.05$) linear or curvilinear regression trends. The calculati of volume-weighted averages is described elsewhere[20]. The h togram in *a* shows annual precipitation values at HBEF.

Both concentration and deposition of SO_4^{2-} at HBEF sl statistically significant ($P \leq 0.0001$ and $P \leq 0.03$) linear decl over the 20-year period (Fig. 1*a*, Table 1). The average rate decrease in H^+ concentration and deposition, based on li regression analysis of data from 1964 to 1983, are not statistic different from those of SO_4^{2-} ($0.9 > P > 0.5$; *t*-test). Howeve contrast to SO_4^{2-}, the regression trends for H^+ concentra and deposition (Fig. 1*b*) have significant quadratic terms (0.03 and $P \leq 0.0001$), and are best described by curvili regressions (Table 1). The trends in concentration and dep tion of H^+ show modest increases during the beginning of sampling period followed by sharp declines towards the (Fig. 1*b*), whereas the SO_4^{2-} trends show constant rate decrease throughout the period (Fig. 1*a*).

The absence of a precise correlation between long-term tre in SO_4^{2-} and in H^+ has led some authors to argue that relationship between SO_4^{2-} and precipitation acidity is uncl and that further reductions of SO_4^{2-} concentrations will affect precipitation acidity[5,6]. However, this ignores the co plexity of the chemical relationship between precipitation aci ity and SO_4^{2-} concentration. Decreases in SO_4^{2-} concentratio would result in stoichiometric reductions in H^+ [illegible] only if other strong acid anions and bas[illegible] unchanged. In fact, precipitation [illegible] influenced by significant, and sometimes complex, trends in other strong acid anions (for example NO_3^-), and in basic materials available to neutralize strong acids (Table 1)[21,22]. Thus we would not expect a stoichiometric correlation between the long-term trends of SO_4^{2-} and H^+ in precipitation at HBEF.

For example, although SO_4^{2-} concentration in precipitation decreased at HBEF during the first half of the sampling period (Fig. 1*a*), 1964 to 1975, the contribution of NO_3^- to precipitation acidity increased, and concentrations of base cations and Cl^- decreased. H^+ ion concentration remained fairly constant during this time (Fig. 1*b*), as potential H^+ decreases due to the SO_4^{2-} decline were balanced by trends in the other ions.

Because precipitation acidity at HBEF has also been affected by factors other than SO_4^{2-} trends, the relevant question is not whether trends in H^+ have mirrored decreases in SO_4^{2-}, but rather: 'What would the precipitation acidity at HBEF have been if SO_4^{2-} concentration had not decreased since 1964?'

To answer this question we calculated the acidity of precipitation at HBEF, assuming that SO_4^{2-} concentration did not decrease after 1964, and that all trends in other ions and the amount of precipitation remained as observed during the period 1964 to 1983. Predicted concentrations and deposition of H^+ for each year between 1964 and 1983 were calculated by holding SO_4^{2-} concentration constant at the 1964 regression value (65.8 μequiv. l^{-1}) and adding H^+ ions to the cation fraction to balance the increase in SO_4^{2-}. Each mole of SO_4^{2-} thus corresponds to two moles of H^+, as is expected for the atmospheric conversion of SO_2 if other ions remain unchanged[10]. Other ions and precipitation volumes were kept at their original values for any specific year. Our calculation thus predicts the effect of SO_4^{2-} on precipitation acidity, taking into account the effect of long-term trends of other ions on precipitation acidity.

We found that if SO_4^{2-} concentrations in precipitation at HBEF had remained constant at the 1964 regression value, the H^+ concentration would have been ~73 μequiv. l^{-1} in 1983 (Fig. 2*a*). But because there was an actual decrease in SO_4^{2-} concentration between 1964 and 1983, the measured H^+ concentration in precipitation at HBEF decreased significantly from a regression value of 71 μequiv. l^{-1} in 1964 to 45 μequiv. l^{-1} in 1983 (Fig. 2*a*). Thus without the SO_4^{2-} decrease, the average H^+ concentration in precipitation at HBEF in 1983 would have been nearly two-thirds higher than the actual measured concentration.

The variability in the predicted H^+ concentrations between different years (Fig. 2*a*) is due to the contribution of components other than SO_4^{2-} to the H^+ concentration, and to climatic variability. Although the predicted H^+ concentration shows considerable variability during certain periods of the record (for example 1970-73) and is best modelled by a slightly curved trend ($P \leq 0.05$), there is no significant linear decrease when the whole record from 1964 to 1983 is considered ($P > 0.7$).

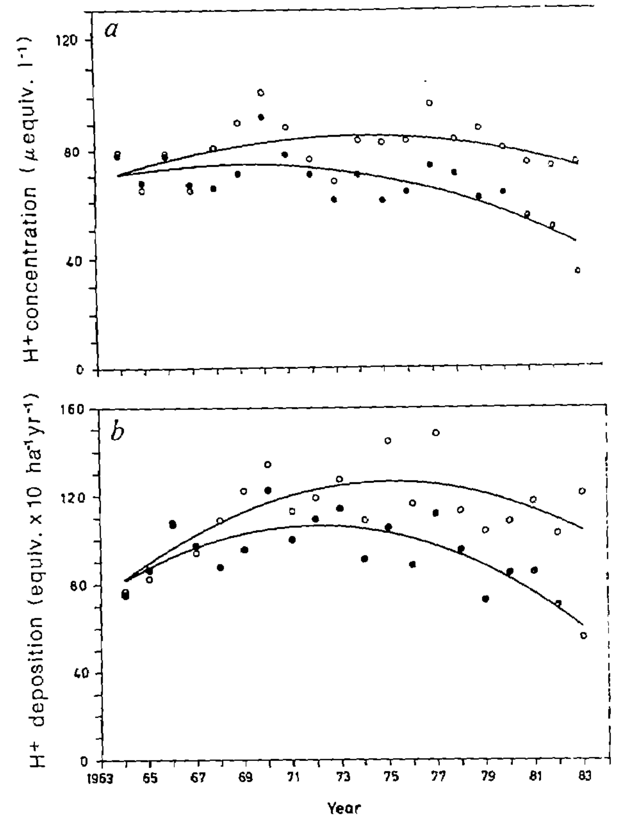

Fig. 2 Actual (●) and predicted (○) volume-weighted average H^+ concentration (*a*) and deposition (*b*) in precipitation at watershed no. 6 of HBEF during 1964–83. The predicted values assume no decrease in SO_4^{2-} concentrations since 1964. Lines represent regression trends. The predicted and actual trends are significantly different ($P \leq 0.01$; *t*-test).

Deposition of H^+ also would have been higher if SO_4^{2-} had not decreased after 1964. If SO_4^{2-} concentrations in precipitation at HBEF remained constant at the 1964 level, H^+ deposition would have increased from a regression value of 820 equiv. ha^{-1} yr^{-1} in 1964 to 1,040 equiv. ha^{-1} yr^{-1} in 1983 (Fig. 2*b*). But the

Table 1 Summary statistics of long-term trends in annual volume-weighted means of ions in precipitation at HBEF

Variable		Linear coefficient	s.e.	$P \leq$	Quadratic coefficient	s.e.	$P \leq$	R^2
SO_4^{2-}	Conc.	−1.49	0.25	0.0001	NS	—	—	0.664
NO_3^-	Conc.	0.0964	0.15	NS	−0.0920	0.028	0.005	0.389
Cl^-	Conc.	−0.723	0.23	0.008	NS	—	—	0.393
H^+	Conc.	−1.36	0.32	0.0004	−0.152	0.061	0.03	0.595
Base cations	Conc.	−1.20	0.20	0.0001	0.108	0.039	0.02	0.714
SO_4^{2-}	Depos.	−14.3	5.8	0.03	NS	—	—	0.255
NO_3^-	Depos.	3.12	2.5	NS	−1.68	0.48	0.003	0.442
Cl^-	Depos.	−9.98	3.2	0.008	NS	—	—	0.389
H^+	Depos.	−11.1	4.0	0.02	−3.84	0.78	0.0001	0.654
Base cations	Depos.	−12.4	2.3	0.0001	NS	—	—	0.625

Trends in SO_4^{2-}, NO_3^-, Cl^-, H^+ and base cations (sum of Na^+, K^+, NH_4^+, Mg^{2+}, and Ca^{2+}) were modelled both as simple linear and as quadratic egressions. Linear regressions are of the form $Y = bX + a$, where b and a are coefficients, Y is ion concentration or deposition, and X is year. uadratic regressions are of the form $Y = b_1X + b_2Z^2 + a$; where b_1, b_2 and a are coefficients, Z^2 is a quadratic year term, corrected to orthogonality y subtraction of $\bar{X}$ ($Z = (X - \bar{X})$). Regression residuals were inspected. Only significant ($P \leq 0.05$) quadratic terms were included in the final odel. The addition of an autoregressive term with a lag time of one year had little effect on standard errors and significance levels. PO_4^{3-} contributed ss than 2% to total anion activity during 1964–83. The contribution of organic acids to free acidity in rainfall at HBEF is about 2%. Monitoring f Al^{3+} started in 1976. To avoid bias in the base cation fraction, Al^{3+} is therefore excluded from any of our trend interpretations. The average $^{3+}$ volume-weighted concentration during 1976–83 was 1.8 μequiv. l^{-1}.

s.e., standard error of coefficient; NS., not significant at $P \leq 0.05$ level; R^2 refers to model including significant terms only.

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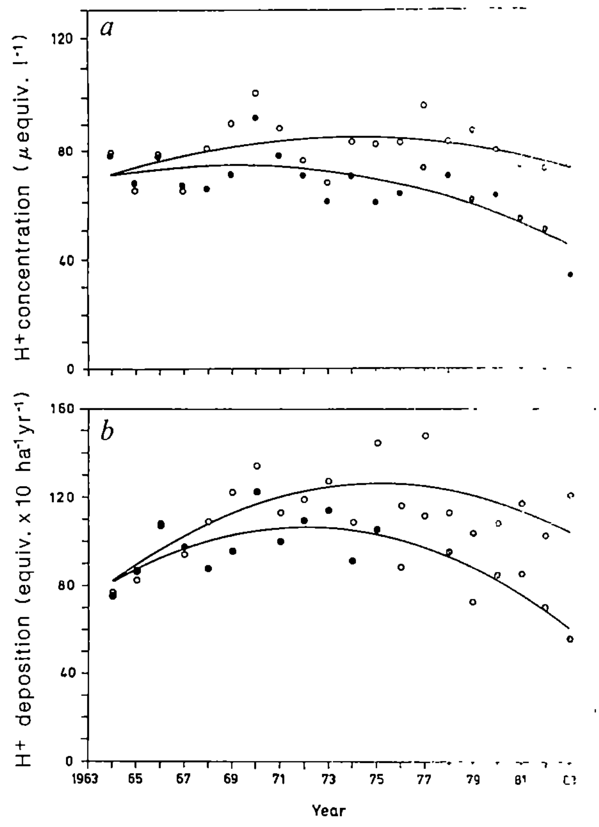

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Cl^-	Depos.	−9.98	3.2	0.008	NS	—	—	0.38[illegible]
H^+	Depos.	−11.1	4.0	0.02	−3.84	0.78	0.0001	0.65[illegible]
Base cations	Depos.	−12.4	2.3	0.0001	NS	—	—	0.62[illegible]

Trends in SO_4^{2-}, NO_3^-, Cl^-, H^+ and base cations (sum of Na^+, K^+, NH_4^+, Mg^{2+}, and Ca^{2+}) were modelled both as simple linear and as quadratic regressions. Linear regressions are of the form $Y = bX + a$, where b and a are coefficients, Y is ion concentration or deposition, and X is year. Quadratic regressions are of the form $Y = b_1X + b_2Z^2 + a$; where b_1, b_2 and a are coefficients, Z^2 is a quadratic year term, corrected to orthogonality by subtraction of $\bar{X}$ ($Z = (X - \bar{X})$). Regression residuals were inspected. Only significant ($P \leq 0.05$) quadratic terms were included in the final model. The addition of an autoregressive term with a lag time of one year had little effect on standard errors and significance levels. PO_4^{3-} contributed less than 2% to total anion activity during 1964–83. The contribution of organic acids to free acidity in rainfall at HBEF is about 2%. Monitoring of Al^{3+} started in 1976. To avoid bias in the base cation fraction, Al^{3+} is therefore excluded from any of our trend interpretations. The average Al^{3+} volume-weighted concentration during 1976–83 was 1.8 μequiv. l^{-1}.

s.e., standard error of coefficient; NS., not significant at $P \leq 0.05$ level; R^2 refers to model including significant terms only.

actual H^+ deposition at HBEF decreased significantly from a regression value of 820 equiv. ha^{-1} yr^{-1} in 1964 to 610 equiv. ha^{-1} yr^{-1} in 1983 (Fig. 2*b*). Without the decrease in SO_4^{2-} concentration, the average H^+ deposition at HBEF in 1983 would therefore have been about 70% higher than measured.

Our calculations assume that long-term trends in different base cations are not influenced by the long-term trend in SO_4^{2-}. This assumption is supported by the fact that base cations in precipitation come primarily from soil dust or from sea-spray rather than from anthropogenic emissions[23]. However, if there were a link between base cation and sulphur chemistry, our results might require some revision. For example, trends of NH_4^+ in precipitation may be directly linked to trends in SO_4^{2-} (refs 23 and 24). But the ratio of NH_4^+ to SO_4^{2-} in precipitation at HBEF varies so widely between different years (max. 0.30; min. 0.13; C.V. = 21%) that this seems unlikely. Yet even if the decrease in NH_4^+ during 1964-83 in precipitation at HBEF were entirely dependent on the decrease in SO_4^{2-}, this would introduce a maximum bias of 5 μequiv. l^{-1} to our calculations.

We conclude that the decrease in SO_4^{2-} content of precipitation at HBEF, generally attributed to decreases in SO_2 emissions, has resulted in large decreases in both concentration and deposition of H^+, thereby diminishing the potential effects of acidification on organisms, water and soil.

Despite decreases, SO_4^{2-} concentrations in precipitation at HBEF remain 4-13 times higher than in remote areas receiving unpolluted rainfall[25]. The regression value for SO_4^{2-} deposition in 1983 (28.5 kg ha^{-1} yr^{-1}) remains larger than the target loadings (14-16 kg ha^{-1} yr^{-1}) suggested as an upper limit for prevention of biological and chemical damage to aquatic ecosystems[24]. Further decreases in SO_2 emissions are therefore likely to decrease SO_4^{2-} deposition and precipitation acidity at HBEF even more.

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Free amino acids in marine rains: evidence for oxidation and potential role in nitrogen cycling

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Previous studies of dissolved organic nitrogen (DON) in precipitation have addressed various aspects of nutrient transport and global nitrogen cycling[1]. In most of these studies however, the detailed chemical composition of DON was not determined. Analyses of specific organic nitrogen compounds within precipitation can yield new information about sources and transformations of DON as well as about heterogeneous oxidative processes in the atmosphere. Dissolved free amino acids (DFAA) are a class of compounds for which surprisingly few analyses in precipitation have been published[1]. We report here the first detailed analyses of DFAA and primary amines in marine rains. Unexpectedly high concentrations of total DFAA were measured, averaging about 6.5 μM (16 marine rain samples) and ranging from 1.1 to 15.2 μM (0.015 to 0.21 p.p.m. N); these values are similar to those obtained for inorganic nitrogen species[2]. Amino acids are predominantly found in rain as their L optical isomers and therefore are most likely biological in origin; however, the exact sources and modes of enrichment in rain over the open ocean are not known. There is evidence that some of the amino acids, in particular methionine, are oxidized in the atmosphere possibly by a heterogeneous photochemical pathway. The finding of high DFAA and primary amine concentrations in marine rain may have important implications in global nitrogen cycling and also may contribute locally to available nitrogen at the sea surface.

Rain, air, and seawater samples were analysed at sea for one year during four recent cruises and at the home laboratory in Miami as part of the SOLARS programme (Studies of Light Activated Reactions in the Sea). These cruises were located in the Gulf of Mexico (RV *Cape Florida*) and in different locations in the northwest Atlantic Ocean (RV *Columbus Iselin*) (Table 1). Amino acids, primary amines and ammonium were analysed on board by high performance liquid chromatography in conjunction with precolumn fluorescence derivatization with *o*-phthalaldehyde and a thiol[3]. For some samples two different thiols, 2-mercaptoethanol (ME) and *N*-acetyl-L-cysteine (NAC), were employed in separate derivatizations. This was done to verify the identification of specific amino acids, since the thiol used in the reaction greatly alters the chromatographic selectivity of the fluorescent derivatives. In addition, the use of NAC results in the separation of L and D enantiomers[4] and also permits more sensitive detection of ammonium than ME. Secondary and tertiary amines cannot be determined by this technique.

Extreme care was exercised to eliminate contamination during sampling. Rain samples were collected with acid washed glass funnels and bottles that were rinsed several times with rain before the actual samples were taken. Air was sampled using an all glass impinger filled with deionized water; the air flow was 1-2 litre min^{-1} and approximately 0.1-0.2 m^3 of unfiltered air were sampled. Most rain samples were analysed unfiltered and within one hour of collection. Several samples were filtered (0.7 μm glass fibre or 0.2 μm membrane) and stored frozen. Filtration and freezing were found not to alter significantly the composition of samples. A summary of the results and background information on a few representative rain and air samples are given in Table 1; detailed DFAA and primary amine compositions of these samples are given in Table 2. Concentrations up to 15 μM were observed in marine rains. Procedural blanks (deionized water or sea water put through the above collection

Table 1 Amino acids, primary amines, ammonium and background data for rain and air samples

Sample	Date	Location	Local Time (EST)	Prevailing wind direction	Amino acids (μM)	Primary amines (μM)	NH_4^+ (μM)	NO_3^- (μM)	Cl^- (μM)	SO_4^{2-} (μM)	H_2O_2* (μM)
				Gulf of Mexico							
B	9/16/85	26°22′ N 89°52′ W	19:30	NNE	13.22	0.10	13.24	18	3,024	191	38.6
D	9/19/85	24°38′ N 82°57′ W	12:17	E	15.17	0.08	ND	ND	ND	ND	20.2
				Miami, Florida							
F	10/2/85	Miami	11:00	SE	0.61	0.25	1.2	282	3,945	366	ND
I-3			17:10	NW	0.39	0.035	3.79	30	82	12	ND
I-3 Hydrolysis			17:10		3.03	1.32	13.14	ND	ND	ND	ND
				Northwestern Atlantic Ocean							
O-3	2/23/86	25°55′ N 78°13′ W	22:55-23:21	SW	10.80	1.36	13.06	1,169	666	52	8.4
Q-1	2/26/86	20°31′ N 68°44′ W	10:25-10:30	N	4.91	0.22	16.36	80	883	70	18.7
T	6/22/86	26°46′ N 75°24′ W	05:45-06:00	E	1.71	0.12	2.81	23	206	ND	ND
U	9/26/86	26°30′ N 76°04′ W	19:00-19:05	E	4.04	0.65	17.52	ND	ND	ND	80
Marine air 1†	2/28/86	13°12′ N 66°04′ W	11:45-16:40	E	2.18	0.022	1.08	527	265	29	ND
Surface sea water (μM)	10/25/86	26°30′ N 76°04′ W	14:40	E	0.062	—	0.42	ND	ND	ND	0.012

ND, not determined.

* See ref. 17.

† Concentrations in nmol m^{-3}.

and sample handling procedures) showed no evidence of amino acid contamination from either the sampling personnel or the ship. Furthermore, the sample handling and derivatization procedures were identical to those routinely used on the same cruises for the determination of DFAA and ammonium in seawater, where total concentrations were 2-3 orders of magnitude lower than in most marine rain samples (Table 2).

The DFAA pattern of marine rain is dominated by glycine, serine, alanine and the acidic amino acids (Table 2). Close examination of the spectra reveals the presence or absence of certain amino compounds that suggest that DFAA are being abiotically degraded in the atmosphere. For example, the absence of methionine in many samples is especially noteworthy, as it is a common constituent of proteins in all organisms. Just as striking is the presence of methionine sulphoxide in nearly all rain and air samples because this amino acid has very few biological sources. Furthermore, methionine sulfoxide eluted as wo peaks corresponding to L-methionine-L-sulphoxide and -methionine-D-sulphoxide. The above results may be xplained in terms of abiotic oxidation of methionine, probably ithin aerosols, resulting in a racemic mixture of the sulhoxides. Previous studies have shown that methionine can be idized by several pathways[5,6] and methionine sulphoxide is major product[6]. We are currently conducting experiments der environmentally relevant conditions to study potential hways and oxidants involved. First results suggest that reac- species produced during the photodecomposition of poten- atmospheric sources of oxidants, such as nitrate and hydroperoxide[7], readily oxidize the reduced sulphur in methion- The high concentrations of primary amines, especially 2- noethanol and methylamine, in several rain samples are also worthy (Table 2). Experiments are being planned to test ether these species can also be derived from abiotic altention carboxylation, for example) of amino acids under the prevail- nditions in marine aerosols. Details of these and related riments will be presented elsewhere.

ith the exception of methionine sulphoxide, only L amino ds were detected, indicating a biological origin for ese mpounds in rain as opposed to abiotic syntheses as sugged by other investigators[8,9]. Although biological in origin, the exact sources, and modes of transport and enrichment of DFAA and primary amines in marine rains are not known at present.

It is evident from Table 1 that marine rains are strongly enriched in DFAA (on average by a factor of ten) relative to terrestrial rains. In fact, it is likely that this enrichment is even more pronounced than our results suggest because rains in Miami also have a marine component. In support of this, Likens *et al.*[10] reported that the mean concentration of total dissolved amines in precipitation at two inland stations was 0.060 μM (about ten times lower than rains collected in Miami). Hydrolysis

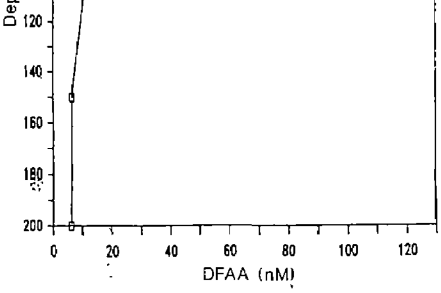

Fig. 1 Effect of rain on the concentration of dissolved free amino acids in the water column at a station in the Gulf of Mexico (27° 10′ N, 89° 02′ W; 9/14/85; RV *Cape Florida* cruise CF8512; station 4). The analyses were performed on board using HPLC, as described in the text. The total DFAA concentration in the rain was approximately 4.5 μM.

Table 2 Free amino acids, primary amines and ammonium in marine rain and related samples (mole % composition and total concentration)

Compound† Amino acid	B‡ (NAC)	D (ME)	F (NAC)	I-3 (NAC)	I-3 (hydrolysed)	O-3 (NAC)	Q-1 (ME)	T (NAC)	U (NAC)	Marine air 1	Surface seawater (NAC)
	Sample* (mole % composition)										
CysA	—	—	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asp	6.2	4.9	0.7	6.7	10.7	5.9	7.7	10.0‖	3.4	8.3	9.8
Glu	4.6	2.4	2.6	13.4	17.2	3.0	6.6		3.8	1.8	7.1
BAGA	tr	—	—	—	—	0.1	—	—	—	—	—
Asn	0.7	0.7	0.5	2.1	—	0.6	0.6	0.6	1.2	1.1	3.2
Ser	24.1	26.4	31.8	13.4	9.4	15.8	15.9	22.3	24.5	26.4	22.4
Gln	0.8	0.2	0.3	2.1	0.4	0.9	0.5	0.6	0.7	1.6	1.6
His	ND	3.9	2.1	ND	—	ND	ND	ND	ND	ND	ND
Thr	5.6	1.8	7.6	4.9	3.2	5.6	2.4	6.2	5.2	3.6	4.0
Gly	18.8	24.4	21.8	15.9	35.7	27.2	17.4	20.0	20.5	22.3	22.6
MetSO	1.8	0.2	—	0.8	0.5	0.7	1.1	0.4	0.3	0.9	—
BAla	—	tr	ND	—	0.5	tr	tr	—	—	0.2	—
Cit	2.9	2.2	0.5	1.8	—	1.1	0.8	2.9	2.5	0.6	1.5
Tau	0.4	0.2	0.5	1.5	0.6	0.3	0.4	0.4	1.7‖	0.4	1.6
Arg	3.0	0.9	0.7	3.9	2.6	0.9	1.0	1.4	—‖	0.9	—
GABA	0.5	ND	0.3	2.1	0.7	0.2	8.4	0.2	0.8	1.1	—
MeHis	tr	ND	—	—	0.1	—	—	—	—	—	—
Ala	8.4	15.2	10.6	10.5	6.0	13.4	10.2	9.4	8.2	10.6	7.3
BABA	—	—	—	—	—	—	—	—	—	tr	—
Tyr	3.1	2.1	ND	2.3	1.1	3.0	1.5	2.2	2.0	0.6	2.9
AABA	—	—	—	—	—	—	—	—	—	1.3	—
Val	3.9	3.4	5.6	4.1	2.5	6.3	2.4	3.4	4.0	3.2	4.5
Met	—	—	—	—	0.1	0.2	0.9	—	0.2	0.7	—
Trp	2.0	0.1	—	0.8	—	0.2	0.3	0.2	0.1	tr	—
Hyl	—	ND	—	—	—	0.7	0.6	0.3	0.4	1.2	—
Phe	1.9	1.3	1.5	2.3	1.5	2.8	1.4	1.6	2.7	2.6	1.3
Ile	2.5	1.9	2.0	2.3	1.6	3.4	1.6	2.6	2.4	2.3	2.7
Orn	3.9	5.0	4.0	3.1	1.0	8.1	4.4	9.4	7.2	3.9	4.0
Leu	4.0	2.0	3.0	4.1	2.6	4.0	2.0	2.2	3.1	3.0	1.6
Lys	2.3	1.4	4.0	2.1	1.8	3.0	2.1	3.5	5.0	1.4	2.3
Total AA (μM)	**13.80**	**15.17**	**0.61**	**0.39**	**3.03**	**10.80**	**4.91**	**1.71**	**4.04**	**2.18§**	**0.062**
2AE	53.0	67.5	57.8	48.6	96.0	93.9	62.7	52.5	69.0	31.8	82.5
MA	47.0	28.9	40.6	40.0	2.0	4.7	28.1	47.5	25.3	68.2	17.5
EA	ND	3.6	1.6	11.4	2.0	1.4	9.2	NA	5.7	tr	—
Total primary amine (μM)	**0.15**	**0.08**	**0.25**	**0.035**	**1.32**	**1.36**	**0.22**	**0.12**	**0.65**	**0.022§**	**0.004**
NH_4^+ (μM)	13.24	ND	1.2	3.79	13.14	13.06	16.36	2.8	17.52	1.08§	0.42

* Sample descriptions for A, B, ... are given in Table 1; ME, 2-mercaptoethanol derivative; NAC, *N*-acetylcysteine derivative.

† Non-standard abbreviations: CysA, Cysteic acid; BAGA, β-aminoglutaric acid; MetSO, methionine sulphoxide; BAla, β-alanine; MeHis, methyl-histidine; BABA, β-aminobutyric acid; AABA, α-aminobutyric acid; Hyl, hydroxylysine; 2AE, 2-aminoethanol; MA, methylamine; EA, ethylamine.

‡ Sample stored at −20 °C.

§ Concentration unit is nmol m^{-3} air.

‖ Asp + Glu coeluted in sample T; Tau + Arg coeluted in sample U.

of unfiltered terrestrial rains (as in Table 2 sample I-3), reveals that the concentrations of combined amino acids and primary amines (principally 2-aminoethanol) are respectively tenfold and fortyfold higher than those of free constituents. Hydrolysis of an unfiltered marine rain on the other hand showed only about twofold and fourfold excesses of combined amino acids and primary amines, respectively, over free constituents. A possible explanation for these results is that land-derived proteinaceous materials (for example, plant fragments, bacteria, yeasts, spores, pollen) associated with dust become leached and hydrolysed within the harsh chemical environment of aerosols as they are transported over the oceans. Expected products would be DFAA, primary amines and ammonium, which then become scavenged by rain. A terrestrial source would be consistent with the findings of other investigators who have shown that other organic constituents (for example, waxes and other lipids) in marine aerosols are in part derived from land sources[11].

Alternatively, the ocean itself may be the main source for the observed enrichment. High concentrations, up to 14 μM protein-N, were reported in marine rains collected in New Zealand and were speculated to be derived from proteinaceous substances in sea spray[12]. In fact, aerosols experimentally generated from seawater have been shown to be enriched by 100–1,000 fold i organic carbon and organic nitrogen relative to bulk se water[13–15]. These aerosols contained 0.5–2.7 mM protein-N while sea foam contained up to 16.8 mM protein-N[12]. Fr these past studies, a marine origin for the high concentratic of DFAA in our marine rain samples seems highly probal However, more sampling over a broader geographical area measurements of parameters such as stable isotopes and DF in size-fractionated aerosols must be accomplished before relative contribution of marine versus terrestrial sources cal estimated.

If the ocean is the major source for the high concentrati of DFAA and primary amines in marine rains, our results sug that these and possibly other organic nitrogen compound: largely recycled over the ocean, although they may be tr ported somewhat inland[12]. On the other hand, if nitroger substances are derived mainly from land sources, then the res imp that there is a net transport of new, biologically utiliza orga c nitrogen to the oceans. In either case, the concentratio of A in marine rains are sufficiently high that they can loc ugment the concentration of available ni sea e and perhaps induce a loca a communicative context in the tests, whereas the naive females learned and responded in a plain instrumental situation not involving intraspecific communication.

Thus, an innate releasing mechanism activated in a communicative context preferentially in the left hemisphere of the brain is the most probable explanation for lateralized recognition of ultrasound in house mice. This interpretation is consonant with those put forward for the left-hemisphere advantage for species-specific call recognition in monkeys[4-6] and with general views of hemispheric laterality in man[2] and animals[12]. It should be considered as a possible basis of the left-hemisphere advantage for speech sound recognition in man.

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A steroid sex pheromone synchronizes male–female spawning readiness in goldfish

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Understanding of pheromone function in teleost fish has been impeded by a lack of information on pheromone identities[1]. Our recent studies[2,3] on goldfish *Carassius auratus*, however, provide strong evidence that 17α,20β-dihydroxy-4-pregnen-3-one (17,20P), the proposed oocyte maturation-inducing steroid hormone in goldfish[4] and other teleosts[5], could be a potent female sex pheromone. Milt (sperm and seminal fluid) volume in goldfish is increased by exposure to 17,20P (and to a lesser extent by exposure to two precursors of 17,20P, progesterone and 17α-hydroxyprogesterone) but not to other steroids proposed as fish pheromones[1,6,7]. In addition, the goldfish olfactory epithelium is extremely sensitive to 17,20P (ref. 3), and the increase in milt volume normally induced by 17,20P exposure is abolished by sectioning the medial olfactory tracts, which previously have been implicated in the control of sex behaviour in male goldfish[8]. We report here that ovulating goldfish release 17,20P into the water and that a rapid (within 15 min) elevation in blood gonadotropin of males mediates the milt response to 17,20P exposure. We conclude that this pheromone system synchronizes milt production with ovulation.

The goldfish is well suited for studies of pheromone release by females and pheromonal effects on males because ovulation is synchronized with the light-dark cycle and can be manipulated under laboratory conditions. Goldfish which have completed vitellogenesis at 12–14 °C ovulate spontaneously within a day of being warmed to 20 °C and exposed to aquatic vegetation, the substrate for oviposition. On a 16 hours light : 8 hours dark photoperiod, the preovulatory gonadotropin (GtH) surge begins after the midpoint of the photophase and induces ovulation (follicular rupture) about 10–12 hours later, during the latter half of the scotophase[9]; oocyte final maturation is completed halfway between the initiation of the GtH increase and ovulation[10]. Spawning begins shortly after ovulation.

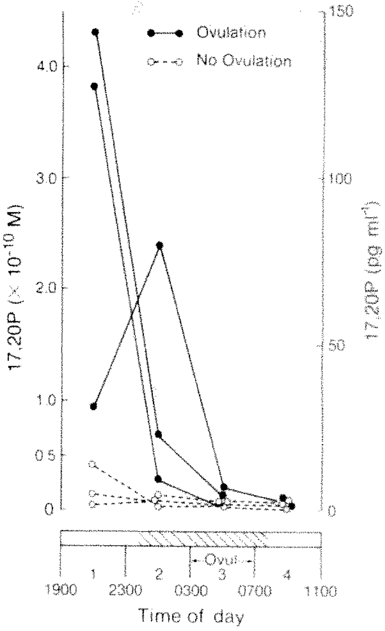

Fig. 1 Concentrations of 17,20P in female holding water during the periovulatory period.

Methods. At 21 : 00 h on day 1, six mature female goldfish (Ozark Fisheries, Stoutland, Missouri) were transferred from stock tanks (14 °C; 16L : 8D photoperiod, lights on at 08 : 00 h) to aquaria (20 °C) containing aquatic vegetation. At 19 : 00 h on day 2, when the preovulatory surge of GtH (ref. 9) should have commenced, females were removed and placed individually in glass jars containing 1 litre of aerated water at 20 °C. At 23 : 00 h, a 10 ml water sample was taken from each jar for 17,20P determination and each female was transferred to a clean jar. This procedure was repeated four times, the last water sample being taken at 11 : 00 h on day 3. Three of the six females ovulated, all during the third sample period (03 : 00–07 : 00 h). Water samples were extracted two times with 2 vol ether and the amount of 17,20P in the extract determined by radioimmunoassay[15,16].

To determine whether or not females release 17,20P, six mature females were maintained individually in 1 litre of water for four successive four-hour intervals encompassing the periovulatory period. Three of the six females ovulated during the third four-hour interval and released high levels of 17,20P into the water during the first and second four-hour intervals (Fig. 1). All water samples from the three females which did not ovulate had relatively low concentrations of 17,20P.

We next used hypophysectomized males to determine whether exposure to 17,20P increases milt volume by stimulating the pituitary-gonadal axis. When hypophysectomized males were stripped of milt[11] two days after surgery and then exposed to a water concentration of 17,20P (5×10^{-10} M) approximating the peak concentrations released by ovulating females (Fig. 1), milt volumes which could be stripped the following morning were significantly ($P < 0.001$) reduced. In contrast, milt volumes of males which had undergone a sham operation and of intact males were significantly ($P < 0.001$) increased following this exposure to 17,20P (Fig. 2).

We determined whether GtH, known to increase milt production when injected into a variety of teleosts including goldfish[12],

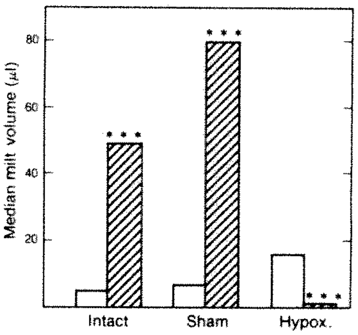

Fig. 2 Effect of hypophysectomy on the 17,20P-induced increase in milt volume in male goldfish. Open bars, initial milt volume; shaded bars, milt volume after exposure to 17,20P. Males were hypophysectomized (Hypox.), had undergone a sham operation (Sham) or were intact (Intact); ***, significantly different from pre-exposure values ($P < 0.001$); $n = 20$ in all groups.

Methods. As in other experiments (Figs 3 and 4), mature male goldfish were maintained before use in flow-through stock tanks at 20 °C on a 16L:8D photoperiod (lights on at 08:00 h). On day 1, males were anaesthetized (0.1% 2-phenoxyethanol, Syndel), then either hypophysectomized by the opercular approach[17], sham operated, or handled without surgery, and placed by groups in 65 l flow-through aquaria (four fish per aquarium) at 20 °C. At 17:00 h on day 3, fish were anaesthetized and stripped of milt to determine initial milt volumes. The fish were placed belly-up in a slotted foam pad and gentle finger pressure was applied to the abdomen to express milt into weighed (±0.1 mg) haematocrit tubes with weighed caps which were then weighed to determine the weight of milt. Milt density was assumed to be 1.0 and milt values are expressed as volumes (µl) rather than as weights. At 21:00 h, water flow to all aquaria was shut off and 10 µg 17,20P (Sigma) in 0.1 ml of ethanol was added to each aquarium; this dose should have produced a water concentration of ~150 pg ml^{-1} (5×10^{-10} M), although actual water concentrations were not measured. At 09:00 h on day 4, all fish were again anaesthetized and stripped to determine milt volumes following exposure to 17,20P. Within each group, milt volumes before and after 17,20P exposure were compared by the Wilcoxon matched-pairs signed ranks test.

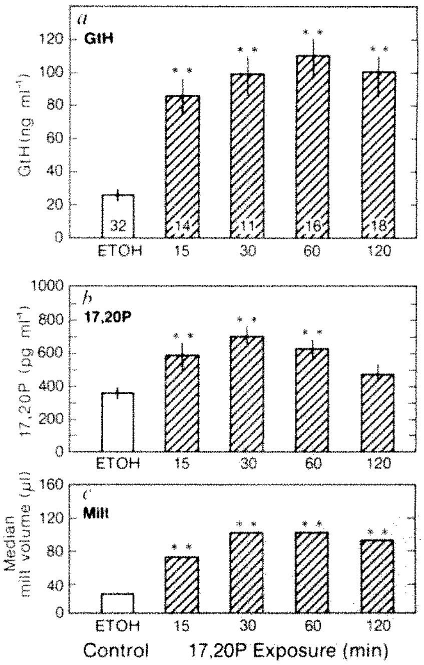

Fig. 3 Effect of water-borne 17,20P on serum GtH (*a*), serum 17,20P (*b*), and (*c*) milt volume in male goldfish. Values in *a* and *b* are means; lines, ±s.e.m. range; **, significantly different from controls ($P < 0.01$). Numbers within bars in *a* show sample size.

Methods. Mature males were placed in 65 l flow-through aquaria (four fish per aquarium) at 20 °C. During scotophase, 10 µg of 17,20P in 0.1 ml of ethanol was added to each experimental aquarium and blood samples were taken from different groups of males under anaesthesia 15, 30, 60, and 120 min later; control males were bled following 60 min exposure to ethanol vehicle. All blood samples were taken during the scotophase (02:00–06:00 h) and all treatment groups were balanced over time. Following blood sampling, all fish were transferred to clean flow-through aquaria and milt sampled 7–8 h after exposure to 17,20P had begun. Serum GtH (ref. 18) and 17,20P (refs 15,16) concentrations were determined by radioimmunoassay. Data on GtH and 17,20P were analysed by ANOVA and Newman–Keuls procedure. Milt volume data were analysed by Kruskal–Wallis ANOVA and Mann–Whitney *U* tests.

increases in the blood of males exposed to water-borne 17,20P. We also measured serum 17,20P levels as 17,20P of testicular origin has been shown to mediate the action of GtH on milt production[13]. Experimental males exposed during the scotophase to 17,20P (5×10^{-10} M) for 15, 30, 60, or 120 min had significantly ($P < 0.01$) higher blood GtH concentrations than did control males exposed to the ethanol vehicle for 60 min (Fig. 3*a*). As well, milt volumes stripped 7–8 h after 17,20P exposure were significantly ($P < 0.01$) greater in the experimental groups than in the control group (Fig. 3*c*). Consistent with the hypothesis that endogenous 17,20P mediates GtH-induced milt increase in goldfish, serum 17,20P levels in three of the four groups exposed to water-borne 17,20P were significantly ($P < 0.01$) higher than in control males exposed to ethanol (Fig. 3*b*). It is unlikely that passive uptake of 17,20P from the aquarium water accounts for these differences between experimental and control groups because serum 17,20P levels in all groups were higher than the calculated water concentration of 17,20P.

A pheromone-mediated increase in milt volume could affect reproductive success if the increase occurs prior to ovulation and spawning. We therefore determined the latency of the 17,20P-induced milt response. Males exposed to 5×10^{-10} M 17,20P or ethanol at the onset of the scotophase were anaesthetized either three or six hours later for blood sampling and determination of milt volume. At both sample times, serum GtH levels in males exposed to water-borne 17,20P were significantly higher ($P < 0.01$ and $P < 0.05$, respectively) than those of control males (Fig. 4*a*). However, only at the 6 h sample time were the milt volumes of males exposed to 17,20P greater ($P < 0.05$) than those of controls (Fig. 4*b*). As peak preovulatory release of 17,20P from females occurs more than 4 h prior to ovulation (Fig. 1), the milt response to water-borne 17,20P appears to be rapid enough to allow an increase in milt volume prior to spawning.

The results presented in this and our earlier studies[2,3] clearly show that 17,20P, which promotes oocyte final maturation in goldfish[4] and is released from females prior to ovulation, also increases blood GtH and milt volume in males. Indirect evidence that these physiological responses of males are normally stimu-

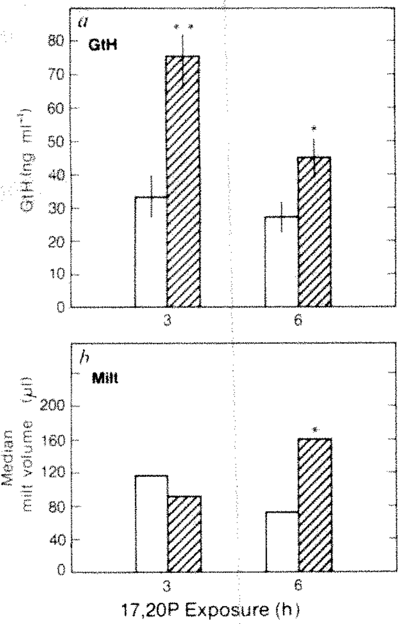

Fig. 4 Effect of 3 and 6 hours exposure to water-borne 17,20P on serum GtH (*a*) and milt volume (*b*) in male goldfish. Values in *a* are means; lines, ±s.e.m. range; **, $P<0.01$; *, $P<0.05$ (significantly different from controls); $n=15$ in all groups. **Methods.** Mature males were placed in 65 l aquaria (three fish per aquarium) at 20 °C. At the onset of scotophase, either 0.1 ml ethanol (open bars) or 10 µg of 17,20P in 0.1 ml of ethanol was added to each aquarium. Three and 6 h later, different groups of males were anaesthetized for blood sampling and determination of milt volume. Data on GtH were analysed by *t*-tests and milt volume data were analysed by Mann–Whitney *U* tests.

lated by females is provided by the recent observation[14] that the presence of an ovulating female goldfish induces a GtH surge in males coincident with that of the female. Both the temporal pattern of 17,20P release from preovulatory females and the latency of the milt response to water-borne 17,20P support our proposal that 17,20P is a pheromone which synchronizes milt production with ovulation. This appears to be the only vertebrate system in which the identity, cellular source and release of the pheromone, as well as the sensory pathway, endocrine mediators and significance of the response in the pheromone recipient, all are clearly established.

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Astrocytes induce blood–brain barrier properties in endothelial cells

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The highly impermeable tight junctions between endothelial cells forming the capillaries and venules in the central nervous system (CNS) of higher vertebrates are thought to be responsible for the blood-brain barrier[1,2] that impedes the passive diffusion of solutes from the blood into the extracellular space of the CNS[3–6]. The ability of CNS endothelial cells to form a blood-brain barrier is not intrinsic to these cells but instead is induced by the CNS environment: Stewart and Wiley[7] demonstrated that when avascular tissue from 3-day-old quail brain is transplanted into the coelomic cavity of chick embryos, the chick endothelial cells that vascularize the quail brain grafts form a competent blood–brain barrier; on the other hand, when avascular embryonic quail coelomic grafts are transplanted into embryonic chick brain, the chick endothelial cells that invade the mesenchymal tissue grafts form leaky capillaries and venules. It is, however, not known which cells in the CNS are responsible for inducing endothelial cells to form the tight junctions characteristic of the blood–brain barrier. Astrocytes are the most likely candidates since their processes form endfeet that collectively surround CNS microvessels[8,9]. In this report we provide direct evidence that astrocytes are capable of inducing blood–brain barrier properties in non-neural endothelial cells *in vivo*.

Astrocytes were purified from neonatal Sprague–Dawley (S/D) rat cerebral cortex as previously described[10]. More than 95% of these cells were labelled by an antiserum against glial fibrillary acidic protein (GFAP), a useful marker for astrocytes in the CNS[11]. All of the GFAP-positive cells had a morphological and antigenic phenotype characteristic of type 1 astrocytes[12]. When a suspension of these cells was injected into the anterior eye chamber of adult syngeneic rats, as described by Olson *et al.*[13], the astrocytes formed aggregates on the surface of the iris and these became vascularized within 48 hours, as observed with a dissecting microscope (not shown). The dye Evans blue, which binds strongly to endogenous albumin[14], was injected intravenously 2 weeks after the injection of the astrocytes in order to assess the 'tightness' of the newly formed vessels in the astrocyte aggregates: although most of the tissues in the rat were stained blue, the astrocyte aggregates remained white (Fig. 1*A*), suggesting that the capillaries and venules in the aggregates were tight. When meningeal cells, prepared from neonatal rats as previously described[15], were injected instead of astrocytes they also formed vascularized aggregates on the surface of the iris, but these stained blue following an injection of Evans blue (Fig. 1*B*), indicating that capillaries and venules in the aggregates were leaky.

These macroscopic findings were confirmed microscopically in frozen sections. Following Evans blue injection, a large amount of the dye, which fluoresces red under UV light[16], was found by fluorescence microscopy in the extracellular space in sections of meningeal cell aggregates but not in sections of astrocyte aggregates, even though both types of aggregates contained small blood vessels (not shown). Similarly, when endogenous albumin was stained immunohistochemically, intense labelling was seen in the extracellular space in sections of meningeal cell aggregates (Fig. 3*D*), but not in sections of astrocyte aggregates (Fig. 3*B*). Thus endothelial cells formed capillaries and venules that were functionally tight in astrocyte aggregates and functionally leaky in meningeal cell aggregates. As expected, in the former aggregates, GFAP-positive astrocyte

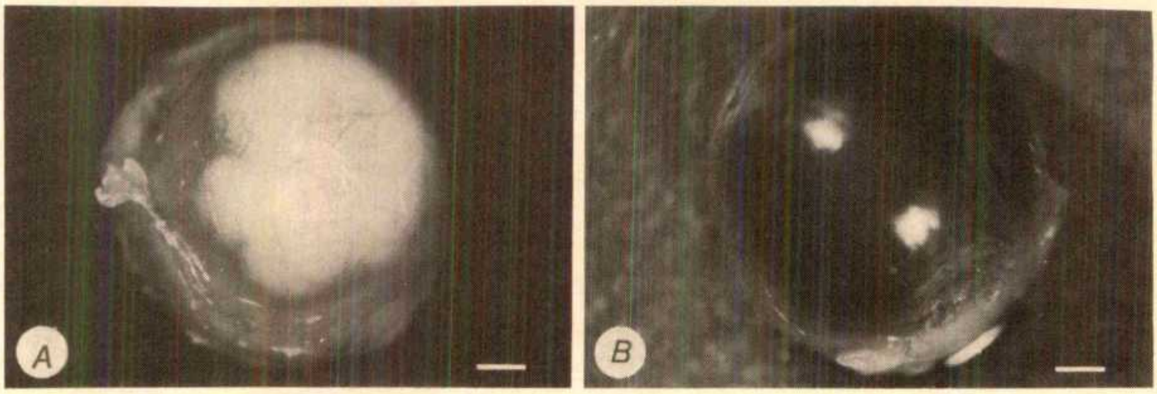


Fig. 1 Vascularized aggregates of astrocytes (*A*) and meningeal cells (*B*) in the anterior eye chamber after injection of Evans blue. The astrocyte cell aggregate in *A* is unstained; the meningeal cell aggregate in *B* is intensely stained. The two white patches in *B* are optical artefacts. Scale bars, 1 mm.

Methods. Rat astrocytes were preapared as previously described[10], using a modification of the procedure of McCarthy and de Vellis[21] as described by Noble *et al.*[22]. In brief, cerebral cortex cultures from 1-2 day old S/D rats were grown in Falcon flasks for 7-9 days in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (DMEM-FCS). The flasks were shaken overnight on a rotatory platform to remove the top cells, mainly neurons and cells of the oligodendrocyte-type-2-astrocyte (O-2A) lineage[23]. The cultures were then treated with cytosine arabinoside (2×10^{-5} M) for 2 days to kill rapidly dividing cells. The cells were then removed from the culture flask with 0.125% (w/v) trypsin and 0.02% (w/v) EDTA (trypsin-EDTA) and recultured in flasks in DMEM-FCS for 7-14 days, after which they were removed with trypsin-EDTA, washed in DMEM-FCS and suspended in DMEM for injection. More than 95% of the cells were GFAP-positive[24]. In some cases, before reculturing, the cells were treated in suspension with monoclonal A2B5[25] and anti-galactocerebroside (GC)[26] antibodies in the presence of rabbit complement as previously described[12] to kill any remaining cells of the O-2A lineage; less than 1% of the cells in such preparations were A2B5-positive or GC-positive. The results obtained were similar with antibody-treated and untreated astrocytes. Meningeal cells were prepared from neonatal S/D rats as previously described[15] and, after five passages, were prepared for injection as described for astrocytes. Recipient rats were anaesthetised with ether and their pupils dilated with 1% atropine-sulphate applied locally. A no. 27 gauge needle was inserted into the posterior eye chamber and left in place during the injection procedure to allow the intraocular pressure to equilibrate. The cells ($0.5-1\times10^6$ suspended in 5 μl of DMEM) were injected slowly into the anterior eye chamber through a glass pipette connected via plastic tubing to a motor-driven microinjection device. Two weeks later, the rats were reanaesthetised and 1 ml of 2% (w/v) Evans blue (Sigma) in Sorensen buffer *p*H 7.2 was injected intravenously. After 2-10 min, the rats were perfused through the ascending aorta with 4% paraformaldehyde in the same buffer containing 7% (w/v) sucrose. The eyes were removed and immersion-fixed in the same fixative. Photographs were taken with a Nikon dissecting photomicroscope using Ilford PF4 film.

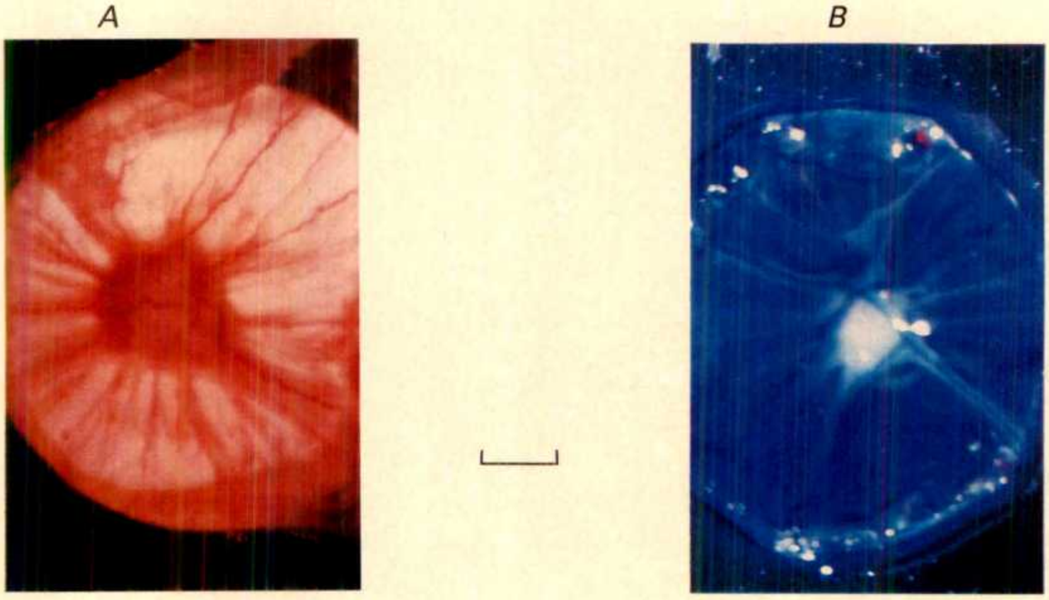
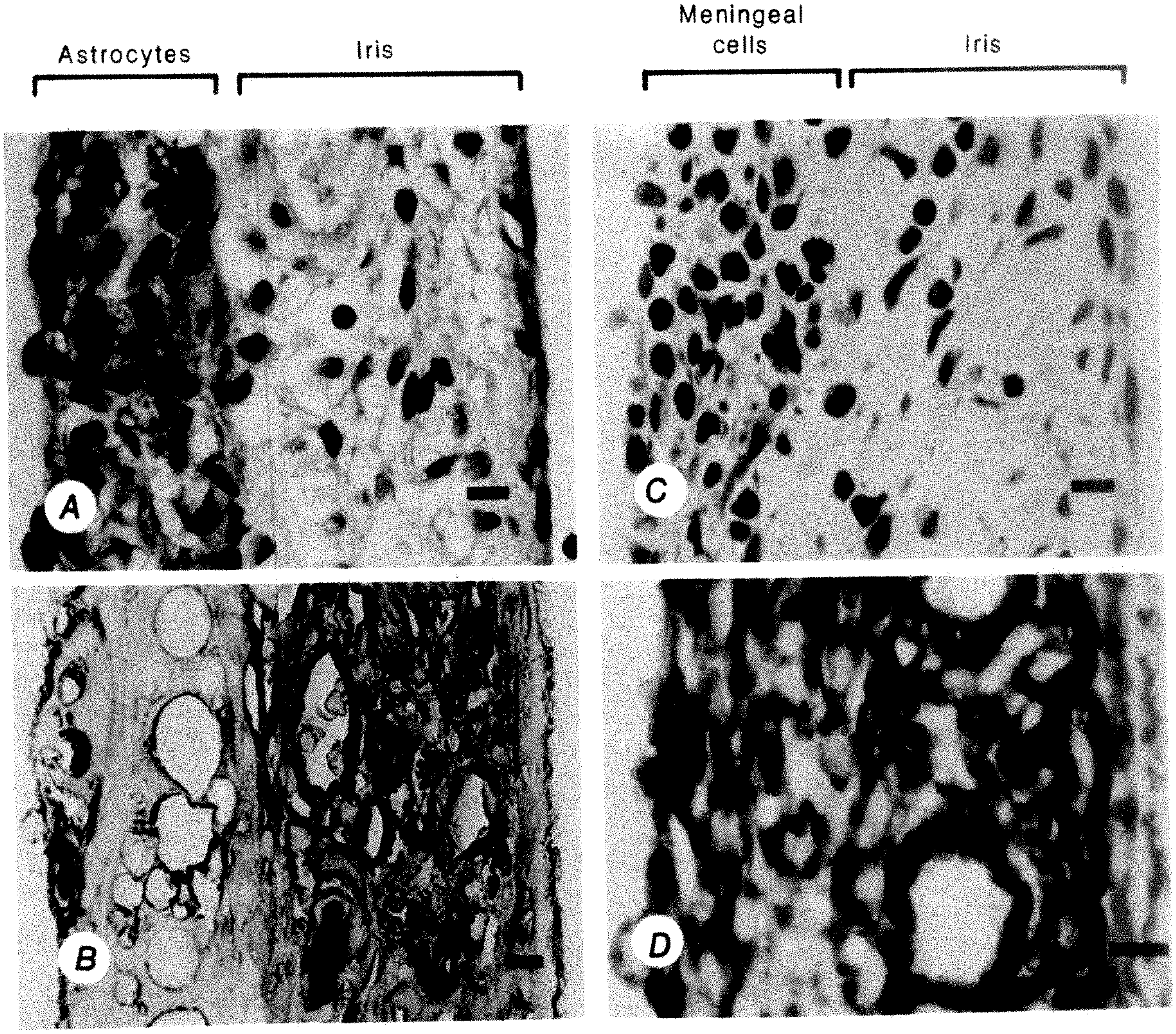


Fig. 2 Vascularized astrocyte aggregates growing on Millipore filters inverted on chick chorioallantoic membranes, *A*, before and *B*, after Evans blue perfusion. Scale bar, 2 mm.

Methods. Astrocytes (5×10^5 cells), prepared as described in Fig. 1, were cultured in a small spot on a Millipore filter (pore size 0.45 μm) for 24 h in DMEM-FCS. The filter was then put cell-side down onto the chorioallantoic membrane of a 5-day-old chick embryo[29]. The egg was sealed with parafilm and reincubated for 11 days. In *A*, the filter with the adherent chorioallantoic membrane and astrocyte aggregate was cut out and photographed. In *B*, the embryo was perfused with Evans blue, as described in Fig. 1, before the filter with adherent tissue was cut out and photographed.



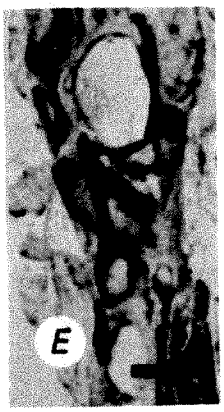


Fig. 3 Immunoperoxidase labelling of astrocyte (*A*, *B*, *E*) and meningeal cell (*D*) aggregates adhering to the anterior surface of the iris, stained with anti-GFAP (*A* and *E*) and anti-albumin (*B* and *D*) serum. A meningeal aggregate adhering to the iris is stained with toluidine blue in *C* to show the extent of the two tissue layers. Note that the astrocyte cell layer is intensely labelled with anti-GFAP serum (*A*) but only very weakly labelled with anti-albumin serum compared with the underlying iris (*B*), but the meningeal cell layer and underlying iris are both intensely stained with anti-albumin serum (*D*). The section in *A* was counterstained with haematoxylin to show the nuclei. GFAP-positive endfeet on small blood vessels in an astrocyte aggregate are shown in *E*. This section was not counterstained. Scale bar, 10 µm.

Methods. Cells were injected into the anterior eye chamber and after 2 weeks the rats were anaesthetised and perfusion-fixed as described in Fig. 1. The eyes were removed, immersion-fixed as described and the anterior poles were embedded in paraffin (*A*) or Epon (*B*, *C*, *D*, *E*). Sections (1 µm) were cut, etched in saturated sodium methoxide[27], and stained by the peroxidase-anti-peroxidase (PAP) method[28]. Before immunostaining, endogeneous peroxidase was inhibited by incubating the sections in 1% H_2O_2 in methanol for 30 min at room temperature. All antisera were diluted in minimal Eagle's medium containing 0.02 M HEPES and 4% calf serum: rabbit anti-GFAP[24] serum was diluted 1:200 for staining paraffin sections and 1:40 for Epon sections; rabbit anti-rat albumin serum (DAKO) was diluted 1:200. Both rabbit antisera were visualized with sheep anti-rabbit immunoglobulin (DAKO, 1:40) followed by rabbit anti-peroxidase complexed with peroxidase (PAP) (DAKO, 1:100) and then 3,3′-diaminobenzidine (DAB) containing 0.005% H_2O_2; the staining was intensified with 2% OsO_4. All incubations were at room temperature for 60 min, followed by three 20-min washes in 0.1 M phosphate buffer, containing 4% calf serum and 0.1% Triton-X-100. The section in *A* was counterstained with haematoxylin and that in *C* was stained with Toluidine blue. Sections were mounted in glycerol-gelatine and photographed with a Zeiss Universal microscope using Ilford PF4 film. In control experiments, no peroxidase labelling was seen when the rabbit antisera were replaced by normal rabbit serum at the same dilution.

endfeet were seen around many small blood vessels (Fig. 3*E*).

The most likely explanation for these findings is that astrocytes induced invading, non-neural endothelial cells to form tight capillaries and venules, but an alternative explanation is that the tight vessels in the astrocyte aggregates were formed by neural endothelial cells that either contaminated the purified astrocyte inoculum or were specifically attracted from the neural retina by the astrocytes. To exclude the latter possibility, rat astrocytes were prepared as above and then cultured at high density in a small area on a Millipore filter for 24 hours. The filters were then placed (cells down) onto the exposed chorioallantoic membrane of 5-day-old chick embryos. After 11 days the chick embryos were injected intravenously with Evans blue: the chorioallantoic membrane turned blue, but the tissue lump formed by the astrocytes remained white (Fig. 2). On the other hand, when filters containing embryonic rat limb fibroblasts

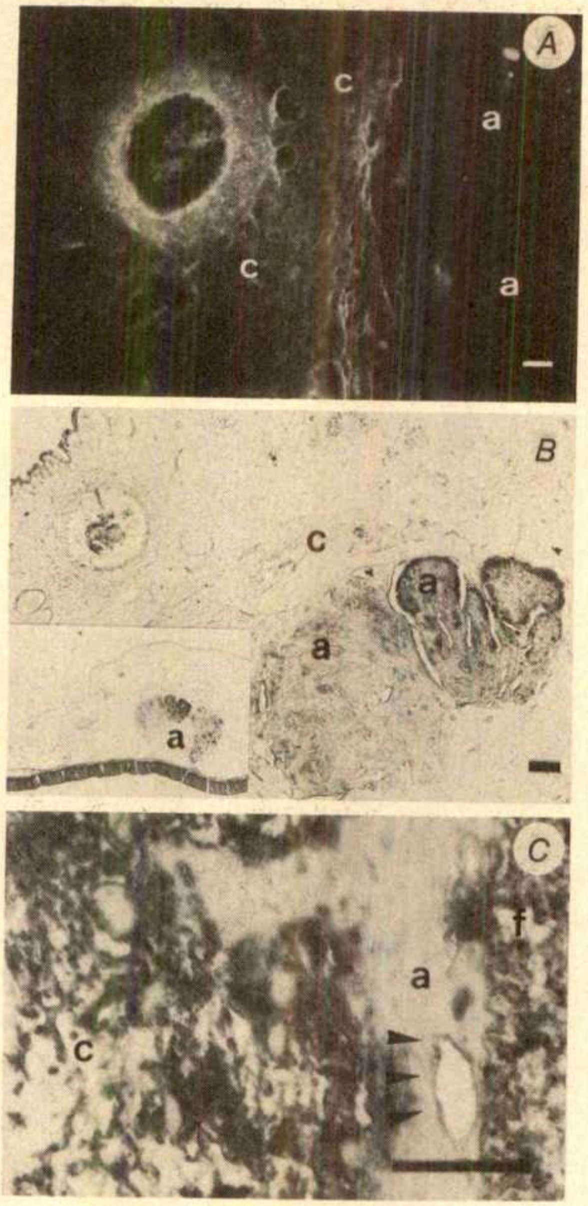


Fig. 4 Fluorescence (*A*) and immunoperoxidase (*B* and *C*) micrographs of astrocyte aggregates growing on Millipore filters inverted on chick chorioallantoic membranes, after Evans blue perfusion (*A*) or staining with rabbit anti-GFAP serum (*B*) or rat anti-chick-cell serum (*C*); *A* and *B* are sections of the same block which is shown as an inset in *B*: the astrocyte aggregate (marked with 'a'), which is GFAP-positive (*B*), is largely unstained by Evans blue, as shown by the lack of fluorescence (*A*), but the underlying chorioallantoic membrane (marked with 'c'), which is GFAP-negative (*B*), is stained by Evans blue, as indicated by the bright fluorescence (*A*). In *C*, all cells in the chorioallantoic membrane are stained with anti-chick-cell serum, while only endothelial cells (marked with arrowheads) are stained within the astrocyte aggregate; the underlying Millipore filter (marked with 'f') is non-specifically stained. Scale bar, 20 μm.

Methods. Astrocytes were grown on Millipore filters inverted on a chick chorioallantoic membrane as described in Fig. 2. After 11 days the filter with the adherent chorioallantoic membrane and astrocyte aggregate was cut out and immersion-fixed at room temperature for 30 min as described in Fig. 1. In *A*, the embryo was perfused with Evans blue before the filter was cut out. The area containing the astrocyte aggregate was cut out and either frozen in isopentane precooled in liquid nitrogen followed by immersion in liquid nitrogen (*A* and *B*) or embedded in Epon (*C*). In (*A*) and (*B*), cryostat sections (10 μm) were cut and thawed onto poly-L-lysine coated glass slides. Immunoproxidase staining was performed using rabbit anti-GFAP serum (diluted 1:100) as described in Fig. 2 (on cryostat sections the endogenous peroxidase blocking step was omitted). For *C*, an antiserum against embryonic chick cells was produced by injecting mechanically-dissociated cells from the liver, spleen, muscles and kidneys of 18-day chick embryos into adult S/D rats. The cells were emulsified with an equal volume of complete Freund's adjuvant and injected both subcutaneously and intraperitoneally. The rats were immunised again in the same way 2 weeks after the first immunisation and were bled 7 days later. When diluted 1:50, this antiserum (using the method described below) stained frozen sections of chick but not rat brain. The antiserum was applied, diluted 1:10 in MEM-HEPES containing 50% (v/v) normal chick serum, to etched, 1-μm Epon sections (prepared as described in Fig. 3). Antibody binding was visualised by the avidin-biotin-complex (ABC) method[17] using a biotinylated rabbit anti-rat-IgG antibody (Vector Laboratories, diluted 1:50) followed by horseradish-peroxidase conmugated ABC (Vector Laboratories). DAB-staining was performed as described in Fig. 2. In all immunostaining experiments, no staining was observed when normal sera were used at the same dilutions as the antisera.

were placed on chorioallantoic membranes, these cell aggregates turned blue after Evans blue injection (not shown). When frozen sections of the astrocyte and fibroblast aggregates were examined by fluorescence microscopy, large amounts of extracellular dye were seen in the chorioallantoic membrane and in the fibroblast aggregates but not in the astrocyte aggregates (Fig. 4*A*). Similar results were obtained when endogeneous albumin in such frozen sections was labelled by indirect immuno-fluorescence with a rabbit antiserum against chick albumin (not shown). To exclude the possibility that the vessels within the astrocyte aggregates growing on chorioallantoic membranes were formed by neural endothelial cells contaminating the cultured astrocyte population, Epon-embedded, etched, 1-μm sections of such aggregates were labelled by the ABC immunoperoxidase method[17] using an antiserum raised against dissociated chick embryo cells. Endothelial cells within the aggregate were intensely stained by the antiserum whereas the astrocytes were not (Fig. 4*C*), indicating that the astrocyte aggregates were vascularized by host-derived chick endothelial cells, rather than by contaminating rat endothelial cells.

Our results provide direct evidence that astrocytes (but not meningeal cells or fibroblasts) can induce endothelial cells of non-nervous system origin to form non-leaky vessels, strongly suggesting that these glial cells are responsible for inducing capillary and venule endothelial cells in the CNS to form the blood-brain barrier. The molecules that mediate this cell-cell interaction must have been highly conserved as they operate between chick and rat cells. In order to characterize these molecules it will probably be necessary to develop *in vitro* models of this interaction. In this regard it is promising that astrocytes can apparently enhance the frequency, length and complexity of tight junctions formed between brain-derived endothelial cells in culture[18] and that cultured glial tumor cells can induce cells of a brain endothelial cell line to express an enzyme[19] and polarized transport[20] characteristic of normal brain endothelial cells.

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Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth

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Angiogenesis, the formation of new capillaries, which is observed in embryonic and injured tissue and is particularly prominent in the vicinity of solid tumours[1], involves the migration and proliferation of capillary endothelial cells. It is probably triggered by agents, such as basic fibroblast growth factor (bFGF), thought to be released from tissues adjacent to proliferating capillaries[1]. As well as being a potent inducer of cell division in capillary endothelial cells *in vitro*, bFGF can act as an angiogenic agent *in vivo*[2]. It is present in a wide variety of richly vascularized tissues including brain, pituitary, retina, adrenal gland, kidney, corpus luteum, placenta and various tumours[1-3]. So far, however, the normal bFGF-producing cell species in these tissues have not been identified[2,4]. We report here that capillary endothelial cells express the bFGF gene, that they produce and release bFGF and that bFGF derived from them can stimulate the proliferation of capillary endothelial cells. We conclude that bFGF can act as a self-stimulating growth factor for capillary endothelial cells, and that it is possible that the formation of new capillaries is induced by capillary endothelial cells themselves.

Initially we found that crude extracts prepared from cultured bovine adrenal-cortex-derived or brain-derived capillary endothelial cells (ACE or BCE cells, respectively)[5], but not from the human leukaemia cell lines HUT-78 or JURKAT[6], stimulate capillary endothelial cell proliferation (data not shown). This suggested that the capillary endothelial cells, but not control leukaemia cells, might contain bFGF.

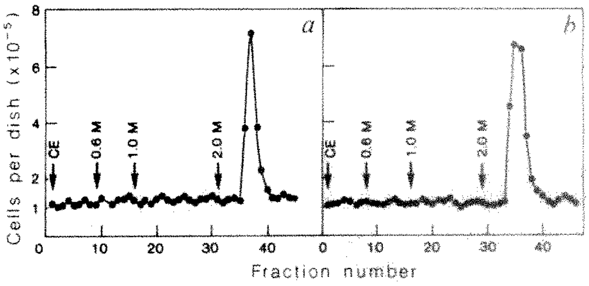

Fig. 1 Heparin-Sepharose (HS) affinity chromatography of crude extracts of (*a*) ACE or (*b*) BCE cells. ACE or BCE cells were maintained in stock plates containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (CS) and antibiotics, and received bFGF (1 ng ml^{-1}) every other day[5]. The cells were passaged continuously as described[5] and used between passages 5 and 16. Confluent cultures were washed with phosphate-buffered saline (PBS), trypsinized, and the cells were centrifuged (300 *g*, 5 min, 21 °C). The pellet was washed again with PBS, and 3×10^7 cells were suspended in 2 ml of 10 mM Sodium phosphate buffer (*p*H 7.4) containing 0.15 M NaCl. Cells were lysed by rapid freezing and thawing followed by passage through a 26 gauge needle. After the NaCl concentration was adjusted to 0.2 M, nuclei and cell debris were pelleted by centrifugation (50,000 *g*, 10 min, 4 °C) and the clear supernatant (referred to as crude cell extract, CE) was applied to an HS column (bed volume, 0.8 ml) that had been equilibrated with 10 mM Tris-HCl buffer, *p*H 7.0/0.6 M NaCl. The column was washed with the same buffer and eluted sequentially with the same buffer that contained 1.0 M or 2.0 M NaCl (refs 8-10), as indicated. The flow rate was 15.6 ml h^{-1}. Fractions (0.52 ml) were collected and assayed for their abilities to stimulate cell growth as follows: fractions were diluted two-fold with DMEM containing 0.5% BSA and 10 µl aliquots of the diluted samples were added every other day to BCE cells that had been seeded at a density of 2×10^4 per dish. After four days, cells were trypsinized and counted in a Coulter particle counter[5]. Values are the means of triplicate determinations which varied by less than 10%.

Subsequently, crude extracts were subjected to heparin-Sepharose (HS) affinity chromatography[7], for rapid and selective purification of FGF[3,8]. All the mitogenic activity present in the crude ACE or BCE cell extracts was retained by HS columns; subsequent elution with 0.6 M, 1.0 M and 2.0 M NaCl yielded a single peak of bioactivity which in both cases eluted with 2.0 M NaCl (Fig. 1*a* and *b*, respectively). This chromatographic behaviour is similar to that reported for bFGF and unlike that of acidic FGF (aFGF), which elutes with 1.0 M NaCl[8-10]. No bioactivity was eluted when crude extracts of the leukaemia cell lines were subjected to HS affinity chromatography (data not shown).

To investigate further the possibility that the capillary-endothelial-cell-derived bioactivity that eluted with 2.0 M NaCl might be bFGF, we examined the pooled, bioactive fractions (fractions 5-7) of the 2.0 M NaCl eluates (referred to as purified extracts) for their abilities to inhibit binding of ^{125}I-labelled bFGF (^{125}I-bFGF) to the FGF receptor[11]. As shown in Fig. 2*a*, purified extracts of both ACE and BCE cells inhibited specific ^{125}I-bFGF binding in a concentration-dependent manner. The abilities of the purified extracts of both cell types to inhibit binding correlated well with their abilities to stimulate the proliferation of ACE cells (Fig. 2*b*) or human umbilical vein endothelial cells (not shown), in spite of the fact that the assays employed differ in their sensitivities to bFGF[2,5,11]. Purified extracts of the leukaemia cell lines neither inhibited specific ^{125}I-bFGF binding (Fig. 2*a*), nor stimulated the proliferation of ACE cells (Fig. 2*b*). Thus, purified ACE or BCE cell extracts contain a bFGF-like mitogen that stimulates the growth of capillary endothelial cells.

The growth-stimulatory activity of the purified ACE cell extract, like that of native bFGF (ref. 12), was completely

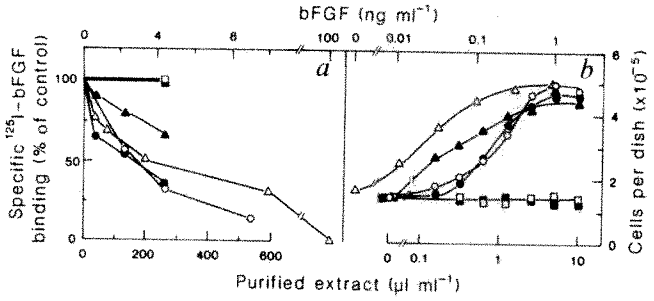

Fig. 2 Effect of HS-purified extracts of capillary endothelial or leukemia cells on (*a*) specific ^{125}I-bFGF binding to baby hamster kidney (BHK-21) cell membranes or on (*b*) the growth of adrenal-cortex-derived capillary endothelial (ACE) cells. *a*, Purified extracts of BCE cells (▲) or ACE cells grown with (●) or without (○) bFGF, and of the leukaemia cell lines JURKAT (■) or HUT-78 (□) or different concentrations of native pituitary bFGF (ref. 8) (△), were examined for their abilities to inhibit specific binding of ^{125}I-bFGF (250 pM) to BHK-21 cell membranes (16 µg of protein)[11]. Specific ^{125}I-bFGF binding, which represents bound ^{125}I-bFGF that was displaced by an excess (100 ng ml^{-1}) of unlabelled bFGF, was determined as described[11]. *b*, The indicated amounts of purified capillary endothelial or leukaemia cell extracts or of native bFGF (symbols as in *a*) were added every other day to ACE cells that had been seeded at a density of 2×10^4 per 35 mm dish. After 5 days, cells present in the dishes were counted. The results are representative for a total of 5 separately performed experiments. Values in *a* and *b* represent the means of triplicate determinations which varied by less than 10%.

Methods. Capillary endothelial cells were grown to confluency as described above. Leukaemia cells were grown without bFGF in DMEM/Ham's F-12 (1/1) medium containing 10% CS and antibiotics. Capillary endothelial cells were washed with PBS and trypsinized before extraction. Capillary endothelial or leukaemia cells (3×10^8) were collected, lysed, and the lysate centrifuged. Protein from the resulting supernatants (25 mg) was then subjected to HS affinity chromatography and the fractions eluted were analysed for the presence of bioactive material (details in legend to Fig. 1). If present, bioactive material was always recovered in fractions 5–7 of the 2 M NaCl eluate. The pooled fractions (purified cell extract) were stored at −70 °C. Aliquots were dialysed before use in the binding assay.

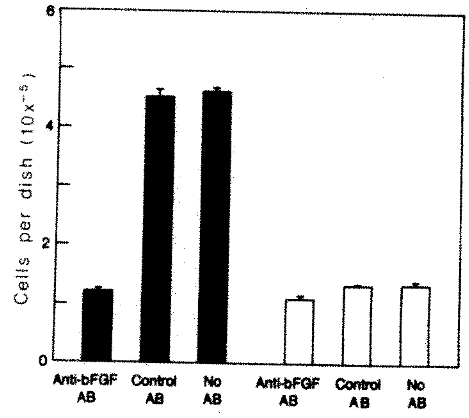

Fig. 3 Effect of specific anti-bFGF antibodies on the proliferation of ACE cells. ACE cells were seeded at a density of 2×10^4 per 35 mm dish and received every other day rabbit anti-bFGF antibodies 'Ede' or rabbit control antibodies (affinity-purified IgG fraction, Flow Laboratories) (6 µg ml^{-1} each) or no antibodies, as indicated, and then either purified ACE cell extract (an equivalent of 1 ng of bFGF ml^{-1}) (solid bars) or no extract (open bars). Values are the means (±s.d.) of triplicate determinations. AB, antibodies.

Methods. Rabbit anti-bFGF antiserum obtained by subcutaneous and intracutaneous injection[21] of pure pituitary-derived bFGF (ref. 8) was purified by protein A-Sepharose affinity chromatography[22]. The antibodies obtained in the IgG fractions ('Ede') recognize bFGF ($ED_{50} = 2$ ng ml^{-1}), but not aFGF, EGF, insulin, transferrin, cytochrome *c* or thyroglobulin (at concentrations up to 0.4 µg ml^{-1}) in a specific radioimmunoassay, where ^{125}I-bFGF is used as the ligand[23]. These antibodies inhibit the bFGF-, but not aFGF-stimulated proliferation of ACE or BCE cells.

destroyed by exposure to heat (5 min at 65 °C) or acidic *p*H (*p*H 2.0, 2 h at 22 °C). Furthermore, the growth-stimulatory effect was completely blocked by anti-bFGF antibodies, but not by control antibodies (Fig. 3, solid bars). Thus, the bFGF-like mitogen present in ACE cell extracts is responsible for the growth stimulation of ACE cells.

Although the anti-bFGF antibodies were able to inhibit completely the proliferation induced by the ACE cell extract-derived bFGF, they had only a small, but consistently observed, effect on the basal proliferation of ACE cells that did not receive exogenous growth factor (Fig. 3, open bars). This suggested that most of the bFGF present in the ACE cells might not be released into the culture medium, but might rather act at intracellular sites, a possibility consistent with the demonstration that the bFGF gene does not code a conventional signal peptide sequence[13]. On the other hand, the existence of specific FGF receptors on the surface of a wide variety of cells (refs 11, 14, 15 and unpublished data) indicates that bFGF must reach the cell exterior by some mechanism. Indeed, bFGF binds with high affinity to basement membrane components such as heparan sulphate proteoglycans, which are known to be deposited on the surface of capillary endothelial and other cells[16]. Thus, bFGF might be bound to heparan sulphate proteoglycans and as such be transported to the extracellular matrix, where it could be active. However, the association of bFGF with extracellular matrix components might mask its immunoreactive epitopes and thereby prevent full neutralization of its bioactivity by the anti-bFGF antibodies. We found that bFGF is indeed present in the extracellular matrix, so that bFGF might be released from these sites into the culture medium.

To assess whether bFGF can be released from ACE cells, serum-free medium (2 l) conditioned by long-term cultures of ACE cells was chromatographed over HS and then analysed as outlined in the legend to Fig. 1. The elution profile was similar to that obtained with the ACE cell extract (see Fig. 1), with all of the bioactivity (~10 pg of bFGF-equivalents per ml of conditioned medium) being recovered in the 2.0 M NaCl eluate (data not shown). Like native bFGF (ref. 11), the material that was eluted with 2.0 M NaCl inhibited ^{125}I-bFGF binding to the FGF receptor and it was able to stimulate the proliferation of ACE cells. Finally, like purified ACE cell extract, the purified conditioned medium lost its ability to stimulate ACE cell growth upon incubation with anti-bFGF antibodies, but not with control antibodies or without antibodies (data not shown). Thus, ACE cells release into the medium a factor indistinguishable from bFGF, that can stimulate their own growth.

The mechanisms by which bFGF is released into the medium are unknown. It is possible, however, that cell lysis or leakage occurring in long-term cultures might be a contributing factor. The existence of similar release mechanisms has been proposed for interleukin-1, another growth factor that lacks a signal peptide[17,18].

The presence of bFGF in extracts and conditioned medium from capillary endothelial cells suggested that these cells express the bFGF gene. Indeed, when poly-adenylated messenger RNA from ACE or BCE cells was hybridized with a bFGF complementary DNA probe[13], two bFGF gene transcripts of 7.0 and 3.7 kilobases (kb) were detected in both cell types (Fig. 4, lanes *a* and *b*, respectively) but not in control cells (Fig. 4, lane *c*), whereas transcripts of the aFGF gene were detected in neither cell type (results not shown). The bFGF gene transcripts are

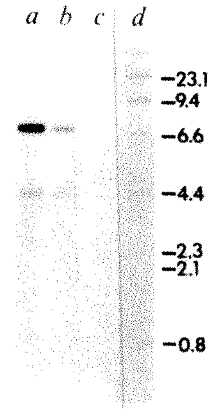

Fig. 4 Analysis of mRNA derived from ACE cells (lane *a*), BCE cells (lane *b*) or HeLa cells (controls) (lane *c*) by Northern blot and hybridization with a bFGF cDNA probe. Lanes *a*–*c* autoradiogram; lane *d*, size markers (kb). Capillary endothelial or HeLa cells were grown in DMEM supplemented with 10% calf serum and antibiotics[5]. Medium from subconfluent cultures was withdrawn and the cells were incubated for 3 days in the same medium containing only 0.5% CS. Cells were then lysed in 5 M guanidinium thiocyanate and polyadenylated mRNA was isolated by oligo(dT)-cellulose affinity chromatography[24]. Aliquots of polyadenylated mRNA from the cells (5 μg per lane, each) were separated on 1.2% agarose-formaldehyde gels[25]. The RNA was transferred to nitrocellulose filters[26] which were probed with a ^{32}P-labelled 1.4-kb *Eco*RI fragment isolated from a bovine bFGF cDNA clone[13]. Expression of bFGF gene transcripts was found in capillary endothelial cell cultures that had or had not received bFGF (1 ng ml^{-1}) (unpublished results) and throughout the time of cultivation (passages 5–16).

identical in size to those previously found in hypothalamus and the SK-Hepl cell line[13], the correct sizes of which are 7.0 and 3.7 kb.

In conclusion, our results demonstrate that capillary endothelial cells produce and release bFGF and that bFGF derived from these cells can stimulate their proliferation in a manner similar to that described for other self-stimulating (autocrine) growth factors[4,19]. Thus, bFGF produced by capillary endothelial cells might contribute to the formation of new capillaries.

That bFGF is present in cells as widely distributed as capillary endothelial cells suggests that it might be accessible to stimulate the proliferation of other capillary-associated FGF target cells, such as vascular smooth muscle cells, fibroblasts, chondrocytes and glial cells, and many other types[2]. We speculate that bFGF derived from capillary endothelial cells may play an important role in embryonal growth and in the maintenance of tissue integrity. In concert with bFGF from activated macrophages[20], it may also participate in the tissue repair mechanisms that start upon inflammation or injury.

The identification of capillary endothelial cells as a source of bFGF might have implications for the understanding of the tumour angiogenesis process, which has previously been thought to be due to bFGF or related factors that are released from the tumours[1]. However, our results suggest that the reverse phenomenon is equally conceivable: tumours might release factors able to stimulate expression, production and release of bFGF in and from capillary endothelial cells and so stimulate angiogenesis. Once the tumours are invaded by the capillaries, local release of bFGF could further enhance the growth of FGF-sensitive tumours.

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A single GTP-binding protein regulates K^+-channels coupled with dopamine, histamine and acetylcholine receptors

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Recently, a GTP-binding protein sensitive to islet activating protein (IAP) has been suggested to be important in producing K^+-currents when the muscarinic receptor of the atrial muscle is activated by acetylcholine (ACh)[1–3]. Here we confirm the blocking effects of IAP and GTPγS (a nonhydrolysable analogue of GTP) on the ACh-induced K^+-current recorded from the ganglion cells of the sea slug *Aplysia* and compare their effects on histamine (HA)-induced and dopamine (DA)-induced K^+-currents. Intracellular injections of IAP irreversibly and selectively block the openings of K^+-channels activated by either ACh, HA, or DA without affecting the resting potential or conductance states of the membranes. Intracellular application of GTPγS alone caused extremely slow, irreversible opening of K^+-channels; however, repetitive receptor activations significantly increase the rate of the GTPγS effect. These results strongly suggest that a GTP-binding protein such as G_i regulates the opening of K^+-channels coupled with these receptors.

Three types of neurons were identified in the abdominal ganglion of *Aplysia*. Under voltage clamp at resting potentials, these neurons responded to ACh, HA, and/or DA with a distinct outward current[4–6], which reversed to an inward current when the membrane was hyperpolarized below the equilibrium potential (−89 mV)[5] for K^+. The observed shifts of reversal potential were in very good agreement with the values predicted from the Nernst equation when the external concentration of K^+ was altered. These responses were always associated with a marked increase in membrane conductance (Fig. 1). Thus all responses to ACh, HA, and DA were produced by an increase in membrane permeability towards K^+.

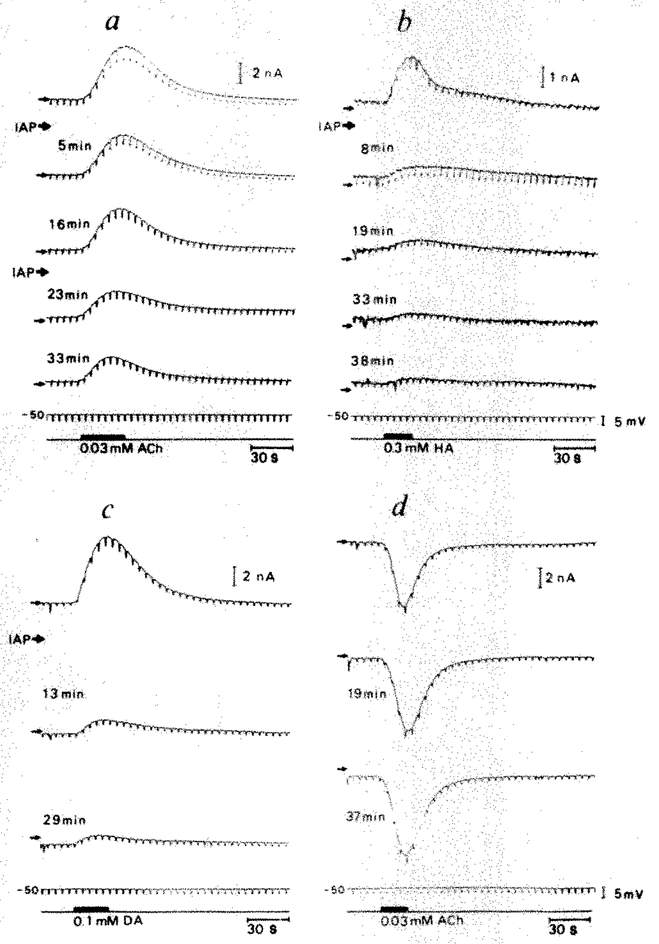

Fig. 1 Effects of IAP on the outward current responses to: *a*, ACh; *b*, HA; *c*, DA; and *d*, on the inward current response to ACh. Data shown in *a*, *b*, and *c* are from 3 different cells but *c* and *d* are from the same cell. Time after the initial intracellular injection of IAP is shown in the left of each trace. Thick arrows, times of IAP injections; thin arrows, initial level of the holding current at the clamped voltage shown (bottom). All cells were clamped at −50 mV. An upward deflection indicates an outward current. Note that the inward current response to ACh was not depressed when the outward current response of the same cell was almost completely blocked by IAP.

Methods. The abdominal ganglion of *Aplysia kurodai* was fixed in a perfusion chamber, and cells were continuously perfused with modified sea water (587 mM Na^+, 12 mM K^+, 671 mM Cl^-, 14 mM Ca^{2+} and 52 mM Mg^{2+}; ref. 7). The effective perfusing volume of the chamber was 0.2 ml. Using a conventional voltage clamp method, the resting membrane potentials were clamped at −48 to −50 mV. The slope conductance of the membrane was continuously monitored by feeding repetitive (0.2 Hz) hyperpolarizing pulses (700 ms, 3–5 mV) to the voltage commander. IAP was dissolved in a solution including 200 mM KCl, 10 mM dithiothreitol (DTT) and 10 mM NAD^+. DTT was used to promote the dissociation of the A protomer from the B subunit[8]. The tip of the microelectrode was filled with a IAP solution of 5 mg ml^{-1} which was applied intracellularly by a pressure pulse of 2–3 kg cm^{-2} with a duration of 200–300 ms. Intracellular concentration of IAP was estimated to be 2–3 μg l^{-1} by calculating a single dose of injection in the volume of the cell. ACh, DA, and HA were dissolved in the modified sea water and applied to the cells by perfusion at a flow rate of 5 ml min^{-1}.

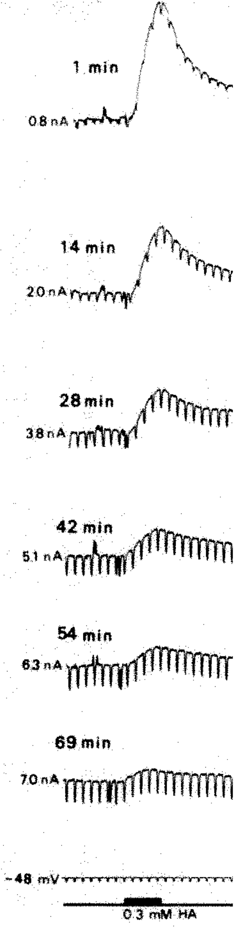

Fig. 2 Effects of GTPγS
responses. Intracellular a
diffusion from the tip of
GTPγS (Li^+ salt). Time
into the cell under study
intracellular concentration
after insertion of the mic
from separate experiments
labelled GTP. The level o
clamped voltage of −48
shown in nA on the left o
in the holding current and
in contrast to the grad

In all cells examined ($n = 36$), intracellular injection of IAP caused no observable change in either the resting membrane potential or conductance, for at least one hour after injection. In contrast, all responses to ACh, HA, and DA were gradually depressed, showing almost a complete block in 40 min after an

injection of IAP that pro
centration of 2–5 μg l^{-1}
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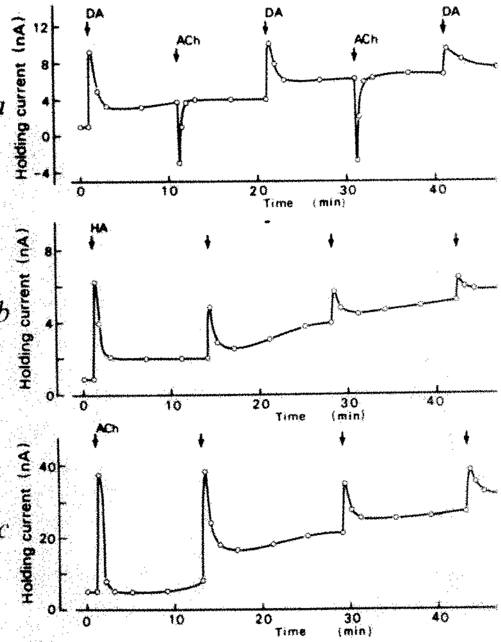
ACh-, HA-, or DA-recep
DA, 12 cells). The effect
the K^+-current produced
neuron shown in Fig. 1*c*
and responded with an
inward current to ACh
current was confirmed to b
that this response to ACh
the K^+-dependent respon
pletely blocked. Similar re
the same types of recepto
5 cells, each of which ha
acid (GABA) and DA. G
were all Cl^--dependent,
dependent. Again, IAP s

Fig. 3 The time courses of the changes in holding currents and responses to: *a*, DA; *b*, HA; *c*, ACh during intracellular application of GTPγS. All cells were clamped at −50 mV throughout the experiment. The cell shown in *a* gave both outward and inward current responses to DA and ACh, respectively. Concentrations of DA, HA, and ACh were 0.1 mM, 0.3 mM, and 0.1 mM, respectively. Application time of each transmitter was 20 s (arrows). Abscissa, time after insertion of GTPγS electrode. Note that the falling phase of the response did not return to the original resting level whenever the response was examined, and that the final level of the holding current at which no response was observable was approximately the same as the peak amplitude of the response recorded at the beginning. *a*, *b*, and *c* are representative results obtained from three different cells with their respective receptors, but similar results were obtained with 27 cells (DA, 12; HA, 5; ACh, 10).



responses without affecting the Cl^--dependent responses, (not shown). All of these results demonstrated that the blocking action of IAP was specific to the opening of K^+-channels coupled with these receptors. This suggested that the binding of transmitter to receptor activates the IAP-sensitive GTP-binding protein, in turn inducing opening of the K^+-channel coupled with each receptor. Inactivation of this GTP-binding protein by IAP should result, therefore, in blocking of K^+-channel opening.

Intracellular application of GTPγS caused a gradual, irreversible hyperpolarization associated with a significant increase in membrane conductance. The time course of this change was extremely slow, normally taking 3 h to reach the maximal hyperpolarization of 20–30 mV. However, this time course was significantly speeded by frequent applications of each transmitter, as shown below. The effects of GTPγS on the transmitter-induced responses were all analysed under the voltage clamp at −50 mV and by recording the current responses associated with the increase in slope conductance of the membranes. Accordingly, the effects of GTPγS on the resting membranes were evaluated only by the changes in the holding current and slope conductance just before applying the transmitters. The effects of GTPγS on the HA-induced responses are shown in Fig. 2. Similar results were obtained from 7 cells with this type of receptor. The apparent amplitude of the current response as well as the conductance response gradually decreased each time when examined repeatedly in the presence of GTPγS. A complete block of the response was seen at 70 min. But the control experiment (single application of the transmitter at 70 min after the onset of GTPγS) showed a much slower rate of increase in the holding current before application of the transmitter (data not shown); however, after the transmitter-induced response, there was a sudden marked increase in the holding current, which remained steady even 3 h after the addition of GTPγS. In the presence of GTPγS, the falling phase of the HA-induced response did not return to the original resting level, leaving a sustained residual response which was not extinguished even after complete removal of HA. Similar effects of GTPγS were seen in the ACh-induced and DA-induced responses (Fig. 3). The residual response accumulated each time the transmitter was applied, and this fact suggested that receptor-activation promoted GDP-GTPγS exchange at the GTP-binding site of the protein, thus speeding the effects of GTPγS. All the K^+-dependent, outward current responses to DA, HA, and ACh

Note that the Na^+-depende
was rather augmented, but
response to DA of the same
The final level of the holdi
same as that of the peak out
to each transmitter. As the
this experiment produced
each cell to each transmitter,
in the presence of GTPγS n
of all K^+-channels coupled
of each cell, resulting in ap
each transmitter. The open
maintained by irreversible
binding site in the α-subuni
preventing the exchange rea
would normally close the K

As shown above, IAP irr
responses to DA, HA, and
presence of GTPγS irreversi
with each type of receptor.
all of these transmitter-ind
were produced by a GTP-
mediator, such as G_i or an

Recently, the outward c
agonists recorded from *locu*
shown to be blocked by I
acute inhibitory effects of m
pig ileum were blocked
responses to opiate- and α
of morphine and clonidine
tion of K^+-channels coupl
or opiate receptors. A num
mones have been shown t
permeability of the recepto
It is highly possible that th
with the receptors of these
ated by IAP-substrates[9,10] s
processes are necessarily d
cyclase. Further evidence is
IAP-sensitive GTP-binding
K^+-channels and that its re
of the change in cAMP, ho
valid only for receptor-cou

-free media or by intracellular
y other investigators[6,15].
d Dr K. Nogimori for their
nd Professor P. Langman for
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n-2
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scribed as a growth factor
n of T cells *in vitro*[1,2] is a
ructure[3] which appears to be
mune responses *in vivo*[4]. As
tes[5] and large granular lym-
(NK cells)[6] can also respond

to IL-2. The action of IL-2 seemed to be limited specifically to lymphocytes, however, and the term 'T-lymphocytotrophic hormone' was used[7]. Here we provide evidence that human monocytes display a substantially increased cytotoxic activity as a direct and rapid response to human recombinant IL-2 but not to human recombinant glycosylated interferon-γ (IFN-γ) or lipopolysaccharide. Our results reveal a previously unknown function of IL-2 and suggest its possible involvement in monocyte-T cell interactions.

Rosenberg and colleagues[8-12] have shown that lymphokine-activated killer (LAK) T cells are induced by treatment with IL-2 in the absence of antigen. These LAK effectors are nonadherent T lymphocytes[9], which very efficiently lyse fresh autologous or allogeneic tumour cells and all cultured tumour cells tested[8-12]. Recently it has been shown that even autologous peripheral blood mononuclear cells are susceptible to their lytic action[13]. In agreement with the data reported by Rosenberg *et al.*[12] and Sondel *et al.*[13], we found that cytotoxic nonadherent T lymphocytes induced by IL-2 (LAK cells) could lyse all established cell lines and all other cells (including normal autologous B cells) that we used as target cells (unpublished

Interleukin 2-induced cytotoxicity in adherent and nonadherent PBM cells

	% Specific lysis				
d	Expt 1 T24 targets	Expt 2a T24 targets	Expt 2b McSa-1 targets	Expt 3a T24 targets	Expt 3b McSa-1 targets
	3.1 (2.7)*	14.2 (3.8)	1.1 (−1.5)	4.7 (1.0)	0.2 (0.1)
	91.8 (7.8)	45.9 (9.8)	52.0 (1.7)	56.4 (5.3)	54.1 (0.4)
	3.0	17.4 (1.9)	1.2 (2.4)	7.8 (4.6)	2.3 (0)
	2.6 (2.7)	5.0 (5.4)	−2.1 (−0.6)	0.2 (−1.4)	0.3 (−0.1)
	67.6 (64.0)	35.0 (33.9)	1.4 (−0.5)	68.8 (66.0)	0.1 (0. 2)
	4.3 (4.2)	6.1 (4.7)	−1.8 (0.3)	3.9 (2.2)	−1.2 (1.4)

isolated by Ficoll-Paque (Pharmacia) density gradient centrifugation were seeded (10^6 cells per well) into
0.2 ml of RPMI 1640 medium supplemented with 2 mM L-glutamine (Sigma), 100 IU ml^{-1} penicillin and
10% heat-inactivated (56 °C, 30 min) autologous or pooled allogeneic serum. After a 2-h incubation under
nidified atmosphere of 95% air and 5% CO_2), the nonadherent cells were removed from some wells by
rred to empty wells and the volume of supplemented RPMI 1640 medium in all wells was adjusted to 0.1 ml
% of PBM cells remained strongly adherent after vigorous pipetting. In experiments 1–3, using cells isolated
ells were strongly adherent cells and 80% were nonadherent. Human IL-2 prepared by recombinant DNA
eversed-phase high performance liquid chromatography[14,26] (500 units per ml) or purified recombinant
nternational Units per ml) were added to some wells and the final volume in wells was adjusted to 0.2 ml.
ibed previously[14]. Endotoxin has not been detected in the purified lymphokine preparations[26,28] using the
a 24-h incubation under standard conditions in the presence or absence of exogenous IL-2 or IFN-γ, cell
nts) was determined using 0.05% trypan blue and radiolabelled T24[16] or McSa-1[30] target cells were added.
:1 for both nonadherent and adherent effectors except for experiment 1 in which the atacker to target cell
ierent and adherent effectors respectively. Some effector cells were treated with CAMPATH 1 and fresh
ore adding radiolabelled target cells and the cytotoxic activity of these lymphocyte-depleted populations is
ysis of target cells was measured using a standard ^{51}Cr-release assay[31]. In brief, after adding radiolabelled
; 5 min) and incubated for 5 h under standard conditions before re-centrifugation and removal of aliquots
ased gamma radioactivity and calculation of the per cent specific lysis. The statistical significance of data
of experiments was evaluated using Student's *t*-test. Differences between control values of lysis (cytotoxicity
id experimental values of lysis (cytotoxicity in the presence of tested substance) were considered to be
P was <0.05. Human IL-2 but not human IFN-γ induced significant cytotoxicity in all experiments.
r cells treated with CAMPATH 1 monoclonal antibody and

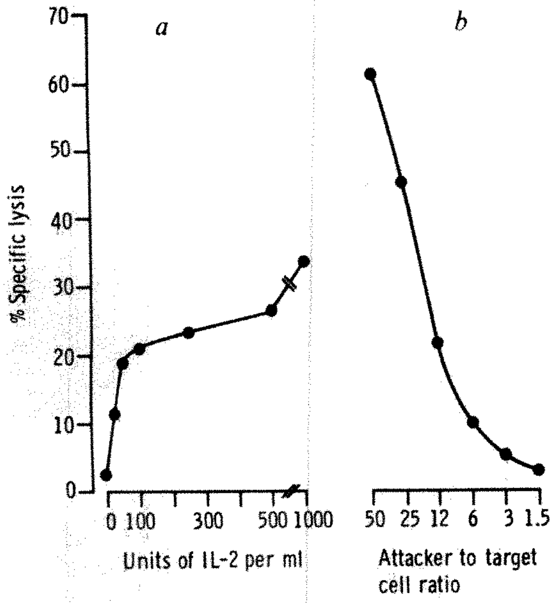


Fig. 1 The dependence of IL-2-induced monocyte cytotoxicity on the concentration of IL-2 and on the attacker to target cell ratio. Monocytes were isolated from human peripheral blood as described[18] and exposed to purified recombinant IL-2 *in vitro* as described in the legend to Table 1. *a*, Cytotoxicity against T24 cells at a 20 to 1 attacker to target cell ratio after a 24-h exposure to different doses of IL-2; *b*, cytotoxicity of monocytes incubated in 500 units per ml of IL-2 at different attacker to target cell ratios. All concentrations of IL-2 (50–1,000 units per ml) induced significant cytotoxicity.

data). During these studies we noticed that human recombinant IL-2 (ref. 14) significantly enhanced the ability of cells in the strongly adherent fraction of human peripheral blood mononuclear (PBM) cells to lyse T24 cells, but not other LAK cell-sensitive targets, for example, McSa-1 cells (see Table 1). Table 1 also shows that the cytotoxic activity of LAK cells was abolished by treatment with an antilymphocyte monoclonal antibody, CAMPATH 1 (ref. 15), and autologous serum as a source of complement, confirming previously published results[9]. However, the cytotoxic activity of adherent cells induced with IL-2 was not decreased by this treatment, indicating that a nonlymphocytic population was involved. The adherent fraction of PBM cells consisted of nearly pure monocytes [up to 93% cells were immunofluorescent when stained with anti-Leu-M3 monoclonal antibody (Becton-Dickinson)]. Because IL-2 had been thought to act only on cells from the lymphocyte lineage (T, B, NK and LAK cells), we examined whether the monocyte cytotoxic activity had been directly induced by IL-2. The target cells used throughout this study were T24 cells, which were derived from a human urinary bladder transitional cell carcinoma and appeared to be relatively resistant to nonspecific NK cell killing[16] but sensitive to monocyte-mediated cytotoxicity[17]. We first investigated the dose response and cell concentration characteristics of this phenomenon, using monocytes prepared by a published method[18] (Fig. 1). The induction of cytolytic activity was directly dependent on the concentration of IL-2 (Fig. 1*a*) and, as in other cytotoxic systems, the percentage of specific ^{51}Cr release was proportional to the attacker to target cell ratio (Fig. 1*b*). But it was possible that the few remaining lymphocytes could activate the monocytes to be cytotoxic through a monocyte cytotoxicity-augmenting factor produced as a response to IL-2. To exclude this possibility, we prepared highly purified monocytes using a solution of

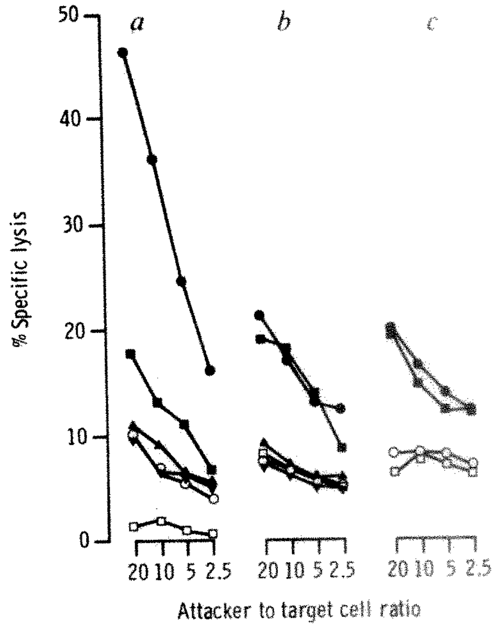


Fig. 2 Effects of lipopolysaccharide (LPS), recombinant glycosylated IFN-γ, and IL-2 on the cytotoxicity of PBM cells and monocytes, with and without treatment with CAMPATH 1 monoclonal antibody plus complement and/or treatment with MHM23 monoclonal antibody. Symbols used in *a*, *b* and *c* are as follows; the percentage of rosette-forming viable cells is given by the first number in parentheses after the symbol; cell viability is given by the second number. *a*, PBM cells: ○ (78%; 90%), cells only; ● (80%; 97%), cells plus IL-2; ▲ (76%; 91%), cells plus IFN-γ; ▼ (77%; 93%), cells plus LPS. *b*, Monocytes: ○ (2%; 94%), cells only; ● (3%, 93%), cells plus IL-2; ▲ (2%; 95%), cells plus IFN-γ; ▼ (2%; 96%), cells plus LPS. *c*, Monocytes pretreated with CAMPATH 1 and autologous serum: ○ (<1%; 92%), cells only; ● (<1%; 92%), cells plus IL-2. □ (all panels), cytotoxic assays performed in the presence of MHM23 monoclonal antibody (cells only); ■, with MHM23 (cells plus IL-2). **Methods.** PBM cells or monocytes were isolated from human peripheral blood using Ficoll-Paque (Pharmacia) or Nycodenz Monocytes (Nyegaard) respectively, according to the instructions of the manufacturers. The resulting cell populations were exposed for 24 h to either purified recombinant IL-2 (500 units per ml) or purified recombinant glycosylated IFN-γ (1,200 International Units per ml) as described in the legend to Table 1 or 50 μg ml^{-1} of lipopolysaccharide from *Escherichia coli* 0127:B8 (Difco). To deplete the monocytic population of residual contaminating lymphocytes before the exposure to IL-2, a part of the monocyte preparation was treated with the rat monoclonal antibody, CAMPATH 1, and autologous noninactivated serum, as described[15]. The purity of monocytes determined by peroxidase stain was >99%. After all treatments, the specific lysis of radiolabelled T24 cells was determined as described in the legend to Table 1. MHM23 (refs 19, 20) ascites fluid was used at a final dilution of 1 in 200 to block lysis and was added to the cells at the time of mixing effectors with targets. In parallel samples, the number of cells forming rosettes with sheep erythrocytes was measured. For rosette assays, mononuclear cells (2.5×10^5 cells in 50 μl) were mixed with 50 μl of washed (in phosphate-buffered saline, PBS) sheep erythrocytes (1% suspension in PBS) and 50 μl of heat-inactivated foetal calf serum, briefly centrifuged (400*g*, 5 min, 4 °C), and incubated overnight at 4 °C. The pellet was gently resuspended in 50 μl of supernatant and was mixed with 50 μl of PBS containing 50 μg ml^{-1} fluorescein diacetate. Viable rosette-forming cells were counted using a Zeiss fluorescence microscope. Samples of some cell suspensions were assayed by immunofluorescent staining before the cytotoxic assay. The monoclonal antibodies used were anti-Leu-11, anti-Leu-M3, anti-Leu-4 (Becton Dickinson) and CAMPATH 1. The percent positive staining, in respective order, for each cell suspension was: untreated PBM cells (6, 21, 66 and 82), IL-2-treated PBM cells (5, 19, 69 and 82), untreated monocytes (<1, 92, <1 and 3), IL-2-treated monocytes (<1, 92, <1 and 3), and IL-2-treated monocytes pretreated with CAMPATH 1 plus autologous serum (<1, 93, <1 and <1). The statistical analysis using Student's *t*-test revealed that IL-2, but not IFN-γ or LPS, significantly augmented cytotoxicity. Moreover, a statistically significant inhibition of PBM cell-mediated cytotoxicity, but not monocyte-mediated cytotoxicity, occurred in the presence of MHM23 monoclonal antibody.

Fig. 3 Reduced cytotoxic activity of effector monocytes following a monocyte-specific lytic treatment. *a*, PBM cells; *b*, monocytes; ○, without IL-2; ●, with IL-2; ■, incubation in medium with IL-2 followed by treatment with anti-Leu-M3 and anti-mouse Ig antibodies plus complement; □, incubation in medium with IL-2 and treatment with anti-Leu-M3 plus complement; △, incubation in medium with IL-2 and treatment with anti-mouse Ig plus complement; ▲, incubation in medium with IL-2 and treatment with anti-Leu-M3 and anti-mouse immunoglobulin (controls for the specificity of complement-mediated lysis).

Methods. PBM cells and monocytes were isolated from human peripheral blood and the monocyte preparation was depleted of residual contaminating lymphocytes by the CAMPATH 1 treatment as described (Fig. 2 legend). Unseparated PBM cells and purified monocytes were exposed for 24 h to recombinant IL-2 (500 units per ml). The cells were then treated with anti-Leu-M3 (a 1:10 dilution, 40 min, 4 °C), washed, treated for 40 min at 4 °C with a 1:10 dilution of rabbit anti-mouse Ig polyclonal antibody (Nordic), washed, and treated with a 1:20 dilution of rabbit complement absorbed with human leucocytes. After all treatments, the specific lysis of radiolabelled T24 cells was determined as described in the legend to Table 1. Before the cytotoxic assay, the IL-2-treated cell preparations were examined by immunofluorescence with anti-Leu-M3 and CAMPATH 1. The percentages of Leu-M3-positive cells and CAMPATH 1-positive cells were 20% and 79% for PBM cells and 92% and <1% for purified monocytes, respectively. The monocyte-specific treatment with antibodies and complement lysed 17% of unseparated PBM cells and 70% of monocytes as judged by Trypan blue staining and caused a statistically significant inhibition of monocyte-mediated cytotoxicity.

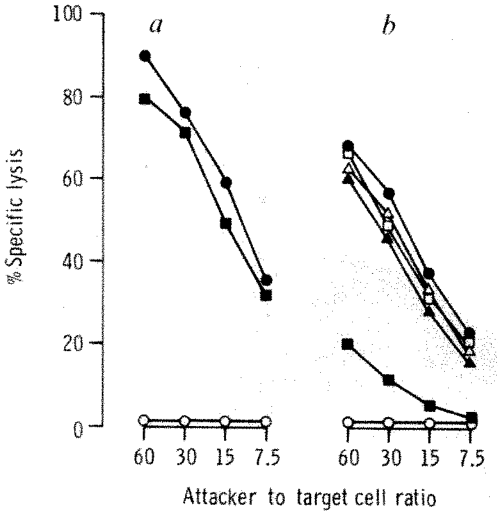
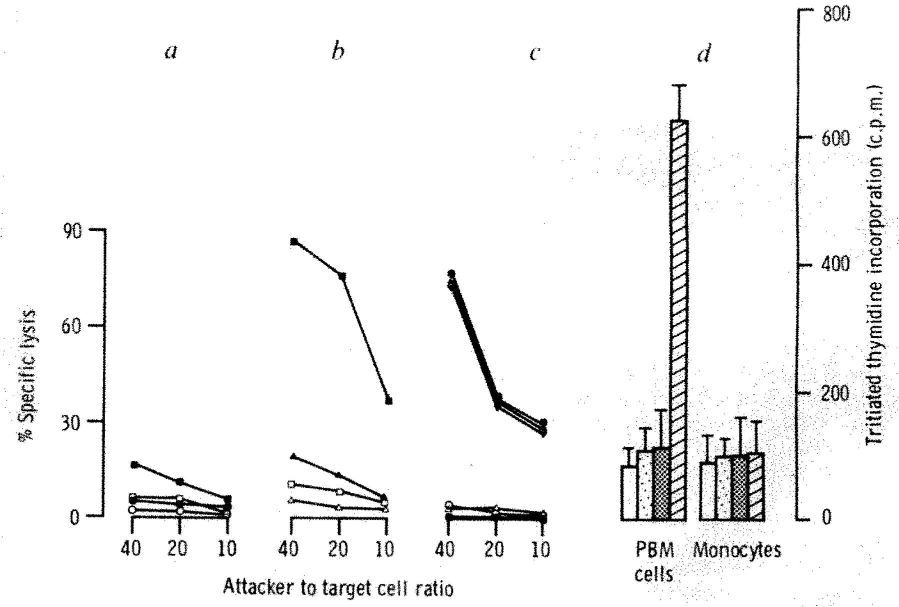


Fig. 4 Induction of monocyte cytotoxic activity by IL-2; proliferation assay and test for mediation by soluble factor. *a*, ■, fresh PBM cells with IL-2; □, PBM cells with no IL-2; ●, fresh monocytes with IL-2; ○, fresh monocytes, no IL-2 (note that the very brief exposure to IL-2 was sufficient to increase the cytotoxic potential of cells, but IL-2 itself had no effect on the chromium release from radiolabelled T24 cells—data not shown). *b*, PBM cells: ▲, treated with IL-2 and CAMPATH 1+ complement; ■, with IL-2, no CAMPATH 1; △, no IL-2, with CAMPATH 1 and complement; □, no IL-2, no CAMPATH 1. *c*, Monocytes: ○, incubated in medium without IL-2; ●, incubated with IL-2; △, treated with CAMPATH 1 after incubation without IL-2; ▲, treated with IL-2 and CAMPATH 1; ▼, treated with CAMPATH 1 before and after incubation in medium with IL-2, and MHM23 present during the cytotoxic assay; ■, T24 target cells exposed to supernatants from monocytes treated with IL-2. *d*, Proliferation assay: □, fresh cells without IL-2; ░, fresh cells with IL-2; ▩, cells incubated 24 h without IL-2; ▨, cells incubated 24 h with IL-2.

Methods. PBM cells and monocytes were obtained from human peripheral blood and exposed to purified recombinant IL-2 (500 units per ml) as described in the legend to Fig. 2. Some samples were treated with CAMPATH 1 and noninactivated autologous serum[15] after the exposure to IL-2. One monocyte sample received the same treatment with CAMPATH 1 before as well as after the expsoure to IL-2 and, in addition, MHM23 monoclonal antibody[19,20] (a final concentration of 1 in 200 ascites) was added just before incubation with radiolabelled T24 cells. The cytotoxicity was assessed following the 24-h incubation with or without IL-2 (*b* and *c*) as well as immediately after the cells were obtained from separation gradients (*a*), also, with or without IL-2 (500 units per ml) present in the assay. Supernatants were taken after 24 h from 0.2 ml cultures of monocytes incubated at concentrations equivalent to attacker to target ratios of 40:1, 20:1 and 10:1, with IL-2 and assessed for the presence of cytotoxic factors by addition to T24 target cells. DNA synthesis (panel *d*) was measured[32] in both freshly isolated cells and after 24 h in culture. Briefly, 10^5 cells were pulsed with 0.5 μCi ^{3}H-thymidine for 5 h and subsequently the incorporation of tritiated thymidine was measured by liquid scintillation counting as described previously[32]. The statistical analysis (Student's *t*-test) of control and experimental values indicated that (1) the IL-2-induced cytotoxicity was significant; (2) the treatment with CAMPATH 1 plus complement significantly reduced the cytotoxic activity of PBM cells; (3) the monocyte-mediated cytotoxicity was not significantly influenced by the treatment with CAMPATH 1 plus complement both before and after the IL-2 induction and by the presence of MHM23 monoclonal antibody; (4) the supernatant from monocytes treated with IL-2 was not significantly cytotoxic for T24 cells; (5) IL-2 induced a significant incorporation of ^{3}H-thymidine into PBM cells, but not into monocytes.



Nycodenz Monocytes (Nyegaard). The monocytes were treated with CAMPATH 1 monoclonal antibody[15] and autologous serum to deplete any residual lymphocytes before exposure to IL-2. In addition, during the cytotoxicity assay, some wells contained MHM23 monoclonal antibody which reacts with the β p95 chain of the LFA-1 molecule and is known to inhibit cytotoxic T cells and NK cells[19,20] (Fig. 2). Neither the treatment with CAMPATH 1 monoclonal antibody, nor the presence of MHM23 monoclonal antibody, nor the combination of both influenced the cytotoxic activity of monocytes. However, the lytic function of unseparated PBM cells was severely diminished by MHM23 monoclonal antibody. These results indicated that the cytotoxic cells were not T lymphocytes or NK cells. Also, neither lipopolysaccharide, nor purified recombinant glycosylated human IFN-γ augmented the cytotoxicity of PBM cells (confirming previous results[21]) or of monocytes (Table 1 and Fig. 2). However, the same preparation of IFN-γ enhanced the expression of major histocompatibility complex antigens on human monocytes (ref. 22 and J. Farrant, personal communication). It is noteworthy that although the dose and batch of lipopolysaccharide we used did not induce any significant monocyte cytotoxicity after a 24-h exposure (8% specific ^{51}Cr release), a very significant enhancement of cytotoxic activity of human monocytes was measured after a 48-h incubation period (57% specific ^{51}Cr release). Cytolytic activity was greatly reduced after monocytes were treated with anti-Leu-M3 (complement nonfixing, first layer) and rabbit anti-mouse Ig polyclonal antibody (second layer) plus rabbit complement (Fig. 3). This treatment lysed 70% of viable monocytes and depleted the cytolytic activity to a similar extent. As previously reported, the cytolytic effect of monocytes against T24 cells did not appear to be mediated directly by a cytotoxic soluble factor[17] or to require DNA synthesis for its induction (Fig. 4). Furthermore, the possibility that an IL-2-induced, PBM cell-secreted factor enhanced the cytotoxicity of monocytes seems unlikely, since the supernatant from PBM cells pulsed with IL-2 had no effect on monocyte cytotoxicity (data not shown). On the basis of these findings, we conclude that monocyte function can be directly influenced by IL-2. Moreover, our data are compatible with a report[23] showing that normal human monocytes express receptors for IL-2 on their surface. These observations, together with a recent study[24], show that IL-2 is not exclusively a lymphocytotrophic hormone. The major physiological role of IL-2 was thought to be activation of resting T lymphocytes (in G_1) to progress through the proliferative phases (S, G_2 and M) of the cell cycle. The biological significance of the effect of IL-2 on monocytes has yet to be determined, but our results imply that the IL-2 system may influence the behaviour of monocytes and their interactions with other cells during an immune response.

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Tumour necrosis factor and lymphotoxin genes map close to *H–2D* in the mouse major histocompatibility complex

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Tumour necrosis factor (TNF-α) and lymphotoxin (TNF-β) are related proteins, secreted by macrophages and lymphocytes respectively, which play a role in destruction of tumour cells and virally infected cells (for reviews see refs 1, 2). TNF-α is a non-glycosylated protein of relative molecular mass 17,000 (M_r 17 K), whereas TNF-β is a glycoprotein of M_r 25 K. Both TNF-α and TNF-β aggregate into multimers and act through the same receptor molecule on target cells. Genes encoding these two TNF proteins have been cloned from mouse and man[3–6] and in both are closely linked, being separated by ~1 kilobase (kb) of DNA[7,8]. In the mouse these genes are located on chromosome 17 (ref. 8), but in man they are on the short arm of chromosome 6 (ref. 9). This segment of chromosome 6 also contains the genes of the major histocompatibility complex (MHC), as does chromosome 17 in the mouse. To find out whether the TNF genes are located within the MHC, we used polymorphic restriction sites to analyse a panel of MHC congeneic and intra-MHC recombinant mouse strains. Initially, we mapped the TNF genes the *D* or *Qa* region in the distal half of the mouse MHC. We then studied a gene cluster encompassing part of the *D* and *Qa* regions and found the TNF genes are located 70 kb proximal to the *D* gene.

A portion of exon 4 of the TNF-α gene was used as a molecular probe to search for polymorphic restriction sites in different inbred mouse strains. Mouse strains AKR, A.SW, CAS3, C3H/HeJ, and C57BL/10 contain a *Bam*HI fragment of ~12 kb, and strains A/J, BALB/c and DBA/2 possess a smaller *Bam*HI fragment of ~10 kb. To confirm that the TNF genes are linked to the MHC, we analysed strains B10.A and B10.D2. The MHC in these mice is derived from strains A/J and DBA/2, respectively, but has been placed on the genetic background of

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Table 1 MHC alleles of inbred and recombinant mouse strains

Strain	MHC donor strains	*K*	*I*	*S*	*D*	*Qa-2*	*Tla*	*Qa-1*	*Hmt*
		Genetic locus							
A/J		k	k	d	d	a	a	a	a
AKR		k	k	k	k	b	b	b	a
A.SW		s	s	s	s	a	b	b	a
BALB/c		d	d	d	d	a	c	b	a
C3H/HeJ		k	k	k	k	b	b	b	a
C57BL/10		b	b	b	b	a	b	b	a
CAS3		c3	c3	c3	w3	a	c3	c3	b
DBA/2		d	d	d	d	a	c	b	a
B10.A	A/WySnSg	k	k	d	d	a	a	a	a
B10.D2	DBA/2	d	d	d	d	a	c	b	a
C3H.OH	DBA/2, C3H/J	d	d	d|	k	b	b	b	a
C3H.OL	DBA/2, C3H/J	d	d|	k	k	b	b	b	a
CAS3(R1)	CAS3, C3H/HeJ	c3	c3	c3	w3	a|	b	b	a
CAS3(R4)	C3H/HeJ, CAS3	k	k	k	k	b	b	b|	b
CAS3(R11)	C3H.SW, CAS3, C3H/HeJ	b|	c3	c3|	k	b	b	b	a

Alleles, based on serological identification, are listed according to refs 10 and 11. Locations of recorded crossovers are indicated by vertical lines.

C57BL/10. The smaller *Bam*HI fragment was found in both these congenic strains (Fig. 1*a*), confirming the linkage of the TNF genes to the MHC.

Next we analysed two congenic mouse strains containing a recombinant MHC derived from two haplotypes distinguishable for the TNF genetic marker. In mouse strains C3H.OH and C3H.OL, the proximal portion of the MHC (oriented towards the centromere) is of DBA/2 origin, whereas the distal portion (oriented towards the telomere) is of C3H origin (see Table 1 and ref. 10). The recombination occurred between *I* and *S* in strain C3H.OL, and between the *S* and *D* region marker loci in C3H.OH. Southern blot analysis of both strains using the TNF-α probe revealed the larger *Bam*HI fragment as in C3H mice and therefore located the TNF genes distal to the *S* region (Figs 1*a*, 2*a*).

We then analysed three intra-MHC recombinant mouse strains, CAS3(R1), CAS3(R4) and CAS3(R11), obtained from matings between C3H/HeJ and CAS3 mice[11]. We found a *Bgl*I fragment of ~23 kb with the TNF probe in CAS3 and a smaller fragment of ~19 kb in C3H/HeJ (Fig. 1*b*). Analysis of the three recombinant strains (Fig. 1*b*) revealed the larger fragment in CAS3(R1) and the smaller one in CAS3(R4) and CAS3(R11). Comparison of this result with the location of the breakpoints and the constellation of the recombinant haplotypes maps the TNF genes distal to the *S* and proximal to the *Tla* region marker loci, within the *D* or *Qa* region of the mouse MHC (Fig. 2*a*).

A 500-kb gene cluster with 13 class I genes, spanning the breakpoint between the *D* and *Qa* regions, has been cloned from the MHC of the BALB/c mouse[12] (Fig. 2*b*). To find out whether this cluster also contains the TNF genes, we analysed cosmid clones constituting the cloned region[12] by dot-blot hybridization. Only cosmid clone II 3.5, located at the proximal end of the cluster[12], gave a positive signal as strong as that obtained from the TNF-α gene used as a positive control (data not shown). This clone was digested with several restriction enzymes and analysed by Southern blot hybridization (Fig. 3*a*). The results, together with a comparison of the restriction maps of cosmid clone II 3.5 (ref. 12) and a mouse TNF genomic clone[8], located the TNF-α and -β genes to a region ~70 kb upstream of the *D* gene (Figs 2*b* and 3*b*). The TNF genes are transcribed in the opposite direction to the class I genes of this cluster.

The MHC, a large genetic region on mouse chromosome 17, encodes class I and class II cell-surface molecules that present foreign antigens to T lymphocytes (for reviews see refs 13, 14). Several class I and class II genes with unknown functions and 6 genes encoding complement components and steroid 21-hydroxylase have been identified in the mouse MHC (Fig. 2*b*). The location of the TNF genes at the proximal end of the *D*/*Qa* region gene cluster is close to genes in the MHC which are also structurally and functionally unrelated to class I and class II genes. It is curious that all these genes (C2/Bf; Slp/C4; 21-OHA/21-OHB; and TNF-α/TNF-β) are organized in pairs of related genes. It is not known whether the tight linkage between genes encoding molecules involved in the recognition (class I and class II) and effector (complement and TNF) phases of the immune response has any functional significance. TNF-α augments transcription of class I genes[15].

In man, certain diseases are associated with the presence of particular MHC alleles in affected individuals (for review see

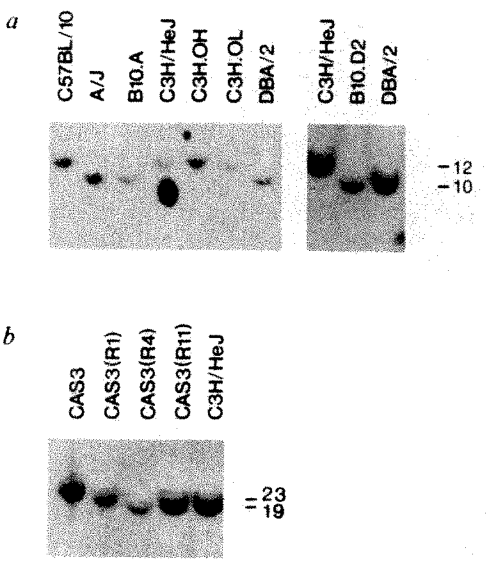

Fig. 1 Southern blot analysis of polymorphic restriction fragments containing the TNF-α gene. *Bam*HI (*a*) and *Bgl*I (*b*) were used to digest liver DNA from the mouse strains indicated. After agarose gel electrophoresis and transfer to Zeta probe membranes[20], hybridization was as described[21] using as a probe an oligolabelled[22] 450-bp *Hin*dII-*Eco*RI fragment derived from exon 4 of the TNF-α gene[8]. Sizes of the TNF-specific restriction fragments (in kb) were determined using restriction fragments of phage λ as markers.

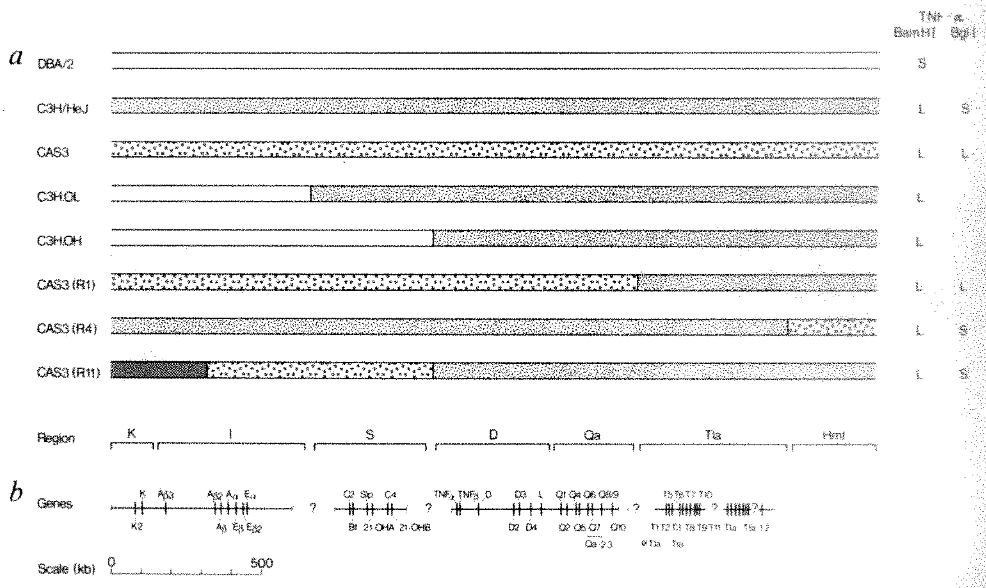

Fig. 2 Location of the TNF-α and -β genes in the mouse MHC. *a*, Polymorphic *Bam*HI and *Bgl*I fragments map the closely linked TNF-α and β-genes to the *D* or *Qa* region of the MHC. Different MHC halplotypes are distinguished by different textures to highlight the origin of MHC DNA in the recombinant mouse strains. Vertical lines, recombinational breakpoints; letters, regions of the MHC defined by them. *Hmt*, a gene which interacts with a maternally inherited factor to determine a cell-surface antigen (Mta), maps between the *Tla* and *Upg*-1 marker loci, about 2 cM distal to *D* (ref. 23) and is separated from *Tla* by the recombinational breakpoint in mouse strain CAS3(R4) (ref. 15). TNF-α alleles, as determined by Southern blot analysis using the restriction enzymes *Bam*HI and *Bgl*I (Fig. 1), are shown as S (small fragment) and L (large fragment). *b*, Gene clusters cloned from the MHC of the BALB/c mouse drawn to scale and identified genes are indicated by vertical lines (additional data from refs 12, 21, 24, 25). Class I genes map to the *K*, *D*, *Qa* and *Tla* regions, class II genes to the *I* region and the complement and 21-hydroxylase genes to the *S* region. ?, Unknown molecular distance between gene clusters. All class I genes of the *Tla* region are located between the recombinational breakpoints in CAS3(R1) and CAS3(R4) (M. Kiefer, K.F.L. and M.S., unpublished data).

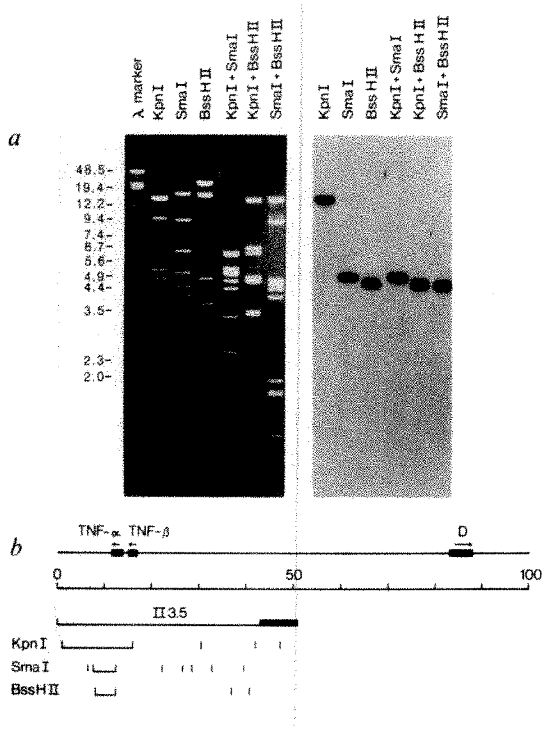

Fig. 3 Location of the TNF genes 70 kb proximal to the *D* gene in the BALB/c mouse. *a*, Fragments resulting from single and double digestions of cosmid clone II 3.5 using several enzymes (*Kpn*I, *Sma*I and *Bss*HII are shown) were separated by agarose gel electrophoresis (left panel), transferred to a Zeta probe membrane and hybridized with the TNF-α probe (right panel) as in Fig. 1. Sizes of phage λ marker framents are given in kb. *b*, Location and orientation of the TNF genes on cosmid clone II 3.5 based on hybridization analysis and restriction map comparison. *Kpn*I, *Sma*I and *Bss* HII restriction sites are shown for the region defined by clone II 3.5 only[12]. Solid box, cosmid vector portion of clone II 3.5; horizontal lines, restriction fragments hybridizing to the probe and therefore containing exon 4 of TNF-α. Orientation of transcription (5'→3') is indicated by arrows. For a complete map of the 500-kb gene cluster see Fig. 2*b* and ref. 12.

ref. 16). From their location in the mouse MHC, the TNF genes are presumably closely linked to the HLA-B gene in man, a location consistent with recent mapping data[17]. Several rheumatic diseases are associated with the HLA-B27 allele, notably ankylosing spondylitis, an inflammatory disease of vertebral joints[16]. It is still unknown whether ankylosing spondylitis is caused by the B27 allele itself or by an allele of a second gene in linkage disequilibrium with B27. TNF-α stimulates collagenase production by human synovial cells[18] and resorption of proteoglycan in cartilage[19]. Thus an abnormal TNF gene could indeed be involved in ankylosing spondylitis and other inflammatory diseases.

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A transcription factor which binds to the enhancers of SV40, immunoglobulin heavy chain and U2 snRNA genes

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In eukaryotes the transcriptional control of RNA polymerase II-mediated gene expression is exerted by *cis*-acting regulatory DNA elements classified as promoter and enhancer sequences. These elements are composed of a number of different protein binding sites (see refs 1, 2 for review). The regulatory factors that recognize such 'modules' may be ubiquitous, tissue- or stage-specific, and positively or negatively acting. According to this model the transcriptional activity of a given gene is programmed by a combination of different modules. We analysed such a site of protein–DNA interaction, the octamer motif[3-6], in the enhancers of the simian virus (SV40) early genes and the murine immunoglobulin heavy-chain gene, and in the distal sequence element (DSE) of the U2 small nuclear (sn)RNA gene of *Xenopus laevis*. The corresponding DNA-binding factor appears to be the same in the three cases. Moreover, a fraction containing partially purified octamer motif binding factor has a stimulatory effect on transcription in an *in vitro* system.

We and others have previously demonstrated stimulation of transcription by the SV40 enhancer in an *in vitro* system derived from cultured cells[7-10]. Accordingly, transcription extracts should contain factors that can bind to enhancer and promoter sequences. To monitor this binding directly, we performed DNase I-footprinting experiments[11] on the SV40 enhancer region (Fig. 1*a*, SV40). Three areas of nuclease protection flanked by DNase-hypersensitive sites could be distinguished in each of the two 72-base pair (bp) repeats within the SV40 enhancer. One centres around the core sequence identified by Weiher *et al.*[13] and the second covers a region referred to here as the octamer motif. Proximal to the 21-bp repeats a third footprint was found which corresponds to the P-box[14]. We observed a similar pattern in the octamer region with extracts from Molt-4 and HeLa cells, and the entire pattern of protection with Molt-4 extract resembled that obtained[15] with HeLa extracts.

The SV40 enhancer represents the prototype of a ubiquitously acting sequence element that stimulates RNA polymerase II transcription in nearly all cell types tested[16]. In contrast, the enhancer of the mouse immunoglobulin heavy-chain gene (IG) is highly tissue-specific[17,18]. When a *Pst*I–*Eco*RI segment which contains most of the IG enhancer activity[17] was taken for DNase I footprinting analysis three major areas of nuclease protection were detected (Fig. 1*a*, IG). These apparent protein-binding sites map to regions that have been found to be insensitive to DMS methylation *in vivo*[19], indicating that the DNase protection observed *in vitro* represents sites of factor–DNA interaction *in vivo*.

The U snRNA genes of *Xenopus laevis* are developmentally regulated[20-23]. Two elements have been identified in the 5′ flanking region of the U2 snRNA gene which are essential for efficient transcription[24]. The distal sequence element (DSE) is located around position −260, and the proximal one (PSE) around position −50, with respect to the RNA start site. The footprint over the DSE is shown in Fig. 1*a*, U2.

Sequence comparison reveals similarities to the octamer motif of the SV40 enhancer in the IG enhancer between nucleotides 540 and 550 (ref. 19) and in the U2 DSE (Fig. 1*b*). Related sites have also been identified in the promoter regions of IG heavy- and κ light-chain genes[3,4], different H2b promoters[25] and U1 and U2 genes[22,26-29].

The IG and U2 enhancers contain 10 bp that are identical (5′ ATGCAAATAG 3′), the first 7 bp also being conserved in SV40, and DNase protection and hypersensitive sites on both strands are found at comparable locations, relative to the common sequence motif, in all three cases (Fig. 1*b*). The possibility that the three DNA domains are recognized by the same component was examined in conditions where specific binding of the octamer binding factor could be detected. Figure 2*a* shows an experiment in which an end-labelled fragment containing the U2 DSE was incubated with nuclear extract and then run on a 5% native polyacrylamide gel. Three bands are seen, corresponding to the free DNA fragment and two species of retarded mobility (x and x′). To prove that the x and x′ complexes resulted from specific binding a combined DNase I-fragment retardation experiment was carried out (Fig. 2*b*). This showed that the region of the retarded bands protected against DNase I digestion correspond to the DSE (compare Fig. 2*b*, lanes 3 and 4, with Fig. 1*a*, U2 and Fig. 4*b*, U2). The protection in the 3′ (upper) region in this experiment is reduced compared to other, similar, experiments and to the protection observed under normal footprinting conditions (Figs 1, 4). Analogous results were obtained with restriction fragments of the SV40 and IG enhancer (not shown) and have also been reported for an (Ig)κ-immunoglobulin chain promoter fragment containing a sequence similar to the octamer motif[5].

We next performed a competition assay to show that the same factor binds to the octamer motifs of the three enhancers. Double-stranded oligonucleotides containing the octamer motif regions of the three enhancers as well as a control oligonucleotide, were used in DNA fragment retardation assays with a fractionated HeLa cell nuclear extract (Fig. 3). All three enhancer oligonucleotides gave rise to complexes with similar or identical mobility (Fig. 3, U2, SV40, IG, lane 0). We then carried out binding assays in the presence of increasing amounts of unlabelled competitor DNA corresponding to either the U2 DSE (Fig. 3, lanes 1–4), the control oligonucleotide, which does not give rise to specific complexes with this fraction of the HeLa cell extract (Fig. 3, lanes 5–8), the SV40 enhancer octamer-motif region (Fig. 3, lanes 9–12) or the IG enhancer (Fig. 3, lanes 13–16). The three enhancer oligonucleotides compete with the labelled probe for complex formation, whereas the control oligonucleotide does not. This indicates that all three enhancer fragments bind to the same factor in the nuclear extract. The SV40 oligonucleotide competes less well than the IG or U2 fragments, suggesting that it has a lower affinity for the factor, perhaps because the SV40 enhancer octamer domain is less similar (Fig. 1*b*).

To purify octamer binding factor further, we used chromatographic fractionation (Fig. 4*a*). As measured by the fragment retardation assay the binding activity is mainly in fraction PC 350 (Fig. 4*a*), which was used for a footprinting experiment The octamer motifs on the three enhancers showed the same protection pattern as with nuclear extracts whereas the other footprints seen with total extract (Fig. 1*a*) were highly reduced, showing that the factor binding to the octamer motif had been enriched relative to the other enhancer binding factors.

The binding activity found in the PC 350 fraction should be correlated with enhancer function. The stimulatory effect of the SV40 enhancer on transcription *in vitro* can be suppressed by increased template concentrations[8], perhaps because of a distribution of promoter and enhancer factors, which have to act simultaneously, to different template molecules. Under these conditions the transcription efficiency should be increased by the addition of enhancer-binding factors only if the template

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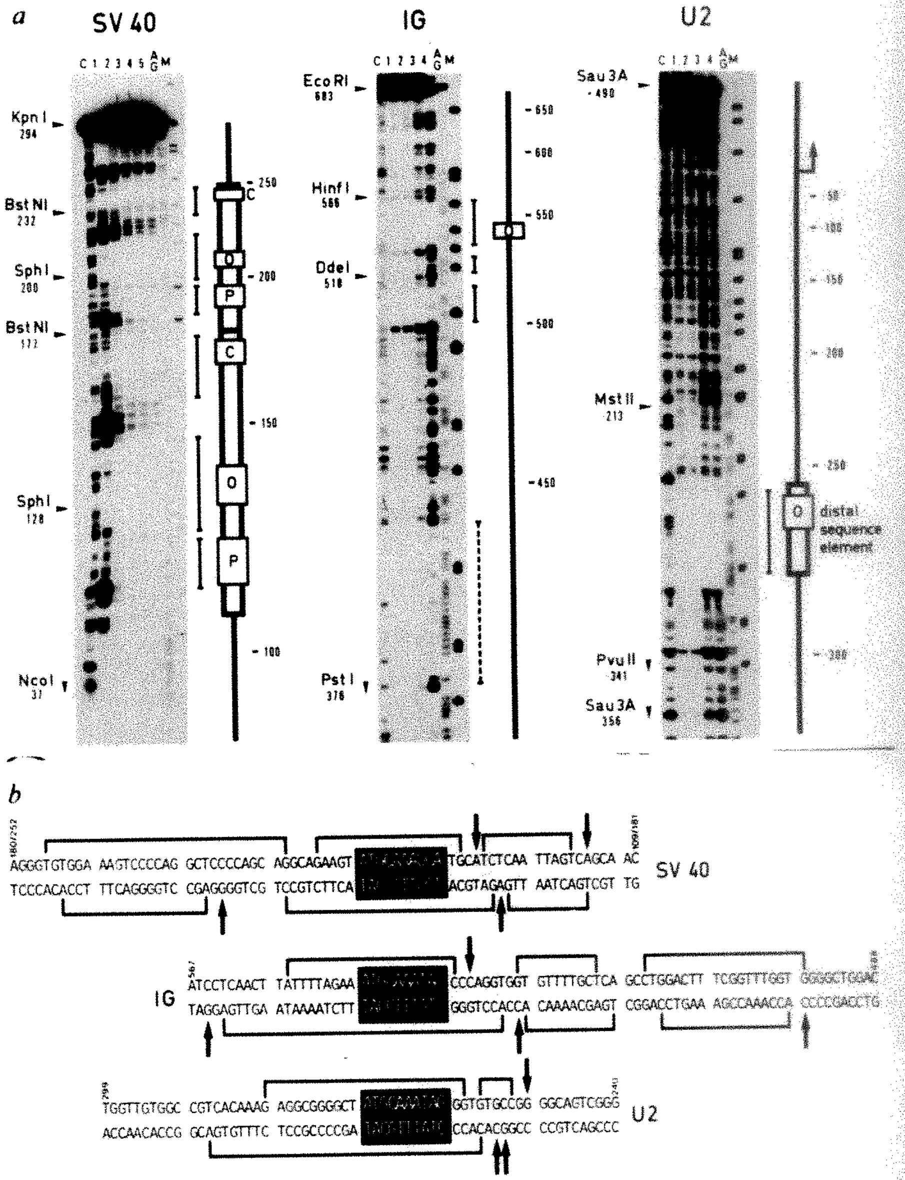
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KpnI 294
BstNI 232
SphI 200
BstNI 177
SphI 128
NcoI 37
250
200
150
100
C
P
O
IG
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EcoRI 683
HinfI 566
DdeI 518
PstI 376
650
600
550
500
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U2
C 1 2 3 4 A G M
Sau3A -490
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PvuII 341
Sau3A 356
50
100
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300
O
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b
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TCCCACACCT TTCAGGGGTC CGAGGGGTCG TCCGTCTTCA ACGTAGAGTT AATCAGTCGT TG
IG
567
ATCCTCAACT TATTTTAGAA CCCAGGTGGT GTTTTGCTCA GCCTGGACTT TCGGTTTGGT GGGGCTGGAC
TAGGAGTTGA ATAAAATCTT GGGTCCACCA CAAAACGAGT CGGACCTGAA AGCCAAACCA CCCCGACCTG
U2
TGGTTGTGGC CGTCACAAAG AGGCGGGGCT GGTGTGCCGG GGCAGTCGGG
ACCAACACCG GCAGTGTTTC TCCGCCCCGA CCACACGGCC CCGTCAGCCC

Fig. 1 *a*, DNase protection on SV40, IG and U2 enhancer and promoter fragments in unfractionated Molt-4 nuclear extract. Digestion products of control reactions without protein (C) and footprinting reactions (1-5) were run together with an A+G-specific sequencing lane (A+G) and an end-labelled *Hpa*II digest of pBR322 (M). Different DNase I dilutions were added for digestion (see below). Important restriction sites are indicated on the left of the autoradiographs. Footprints detected and descriptions of the end-labelled DNA fragments are shown diagrammatically on the right: P, P-box; O, octamer motif; C, enhancer core. SV40 (early coding strand): The 258 nucleotide (nt) *Nco*I-*Kpn*I fragment[32] from position 38 to 294 was 5' labelled at the *Nco*I site with [γ-^{32}P]ATP. IG (non-coding strand): the fragment was 5'-labelled 46 bp away from the *Pst*I site at position 376 in a polylinker *Hin*dIII site and cut at the *Eco*RI site at position 683. Although the footprint between position 400 and 450 on the IG enhancer is not very obvious with this fragment, experiments with DNA fragments from this region labelled at different sites confirmed its presence (not shown). U2 (non-coding strand): the fragment was labelled at a polylinker *Eco*RI site 10 nt away from the *Sau*3A site at position −359 and cut at a polylinker *Pst*I site downstream of the gene. *b*, Comparison of the DNase I protection patterns around the octamer motif homologies in the SV40, the IG and the U2 enhancers. A schematic representation of footprinting experiments performed with different DNA fragments (coding and non-coding strands) is shown. Related sequence motifs (white letters) are positioned above each other. Brackets, protected areas; arrows, DNase hypersensitive sites. Numbers at the boundaries, position of presented sequences.

Methods. Nuclear extracts were prepared according to Dignam *et al.*[33] with modifications[9]. A+G sequencing reactions followed the standard protocol[34]. Nuclear extract (4 µl, 33 µg protein) was preincubated with 25 ng of linearized pUC plasmid DNA on ice for 15 min. Reactions (10 µl) contained 10 mM HEPES-KOH (*p*H 7.9), 20 mM KCl, 4 mM $MgCl_2$, 4 mM spermidine, 0.1 mM EDTA, 0.25 mM DTT and 10% glycerol. End-labelled DNA (10,000 c.p.m.) was added in 2 µl H_2O and incubated for 10 min at 20 °C. DNase I (2 µl, Worthington DPFF), freshly diluted in the incubation buffer to concentrations between 50 and 800 µg ml^{-1}, was added and the reactions incubated for 90 seconds at 20 °C. The DNase concentrations were: Controls without extract (C lanes), 100 µg ml^{-1}; SV40 lane 1, 800 µg ml^{-1}; lane 2, 400 µg ml^{-1}; lane 3, 200 µg ml^{-1}; lane 4, 100 µg ml^{-1}; lane 5, 50 µg ml^{-1}; IG and U2 lane 1, 100 µg ml^{-1}; lane 2, 200 µg ml^{-1}; lane 3, 400 µg ml^{-1}; lane 4, 800 µg ml^{-1}. In control reactions, extract was substituted by incubation buffer. DNase digestion was stopped by addition of 100 µl phenol/chloroform/isoamylalcohol (25:24:1) and 90 µl of 0.4 M LiCl containing 10 µg transfer RNA. The aqueous phases were re-extracted with chloroform/isoamylalcohol (24:1), ethanol precipitated and loaded onto 6% acrylamide-urea sequencing gels.

contains a functional enhancer. Truncated *β*-globin templates either with (CAJ 6) or without (CAD, ref. 7) the SV40 enhancer were used for *in vitro* transcription (Fig. 4*c*). With high template concentrations we observed only a modest (twofold) enhancer activity (Fig. 4*c*, lanes 1 and 2). After addition of fraction PC 350 the amount of correctly initiated globin RNA was increased significantly only with the enhancer-containing template CAJ6 (Fig. 4*c*, lanes 3-6), resulting in an eightfold enhancer effect. Thus fraction PC 350, which contains the octamer-binding factor, does indeed stimulate transcription in an enhancer-dependent fashion. Although at this stage the factor is far from pure, it is likely that the DNA-binding activity and the factor stimulating enhancer-dependent *in vitro* transcription are the same, although additional enhancer binding factors are probably required to obtain the maximal *in vitro* effect.

The fact that the octamer motif was found to be part of the control regions of different transcription units such as the SV40, IG and U2 genes and that it interacts, at least *in vitro*, with a common factor in these three cases indicates that this putative module cannot by itself explain the different regulatory characteristics of the three genes controlled by these enhancers. To explain how the different regulatory programmes are effected, additional steps in the control pathway must be assumed. In the case of the cell-specific IG expression there are indeed indications of tissue-specific factors binding to certain parts of the enhancer (ref. 30 and H.S. and D.B., unpublished data). Interactions with such proteins could then alter either the binding specificity or the transcriptional function of the octamer-binding protein. Alternatively, the octamer-binding factor might not work ubiquitously, being modified or replaced by a related, but cell- or stage-specific factor, necessary to allow proper functioning of regulated enhancers.

Singh and co-workers[5] have demonstrated the presence in nuclear extracts from B cells and HeLa cells of a binding protein

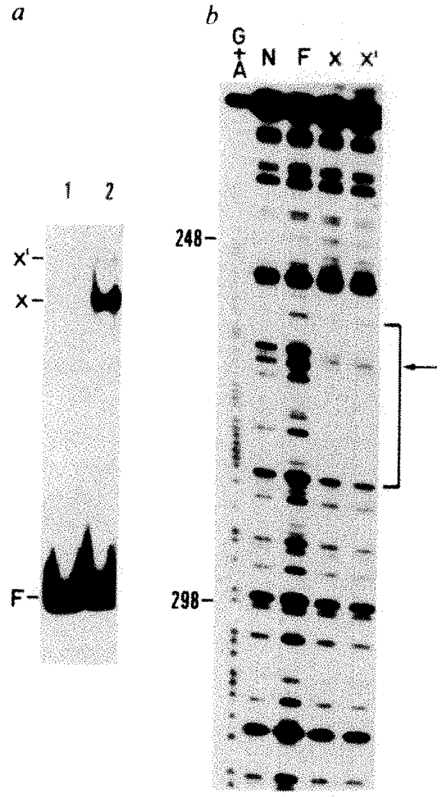

Fig. 2 Analysis of HeLa protein–U2 DSE complexes. *a*, A 5′ end-labelled 150-bp *Eco*RI–*Mst*II fragment containing the U2 DSE was incubated with HeLa nuclear extract, and the resulting complexes were analysed on a non-denaturing polyacrylamide gel. Lane 1, naked *Eco*RI–*Mst*II fragment alone; F, mobility of the free DNA. Lane 2, after incubation with extract two retarded bands, x and x′ are observed. Similar complexes are obtained with fragments from the IG and SV40 enhancer which contain the octamer motif. *b*, Analysis of regions protected against DNase I digestion in the x and x′ complexes. The *Eco*RI–*Mst*II fragment containing the U2 DSE was incubated with HeLa extract, digested with DNase I, then complexes were separated on a non-denaturing polyacrylamide gel. Bands corresponding to F, x and x′ were excised, DNA was eluted and then run on a denaturing polyacrylamide–urea gel. Lane G+A, G+A sequence of the *Eco*RI–*Mst*II fragment; lane N, naked DNA digested with 5 μg ml^{-1} DNase I; lanes F, x and x′, DNA eluted from the corresponding bands. Bracket, region of protection in the retarded fragment; arrow, position of increased DNAse I sensitivity. In other experiments the protection extending upwards from this point was greater. Numbered residues correspond to those shown in Fig. 1*b*.
Methods. *a*, End-labelled fragments (3,000 c.p.m.) containing the putative protein-binding site were incubated together with HeLa nuclear extract (0.5 μl, 4 μg protein) and 1.5 μg poly[d(I–C)] in 15 μl reaction mix. Buffer was 10 mM HEPES-KOH (*p*H 7.9), 100 mM KCl, 4 mM $MgCl_2$, 4 mM spermidine, 0.1 mM EDTA, 0.25 mM dithiothreitol (DTT), 100 μg ml^{-1} bovine serum albumin and 10% glycerol. Samples were incubated at 25 °C for 10 min to allow binding. Loading buffer, (40% glycerol, 50 mM EDTA, 5 × TBE, 0.25% bromophenol blue) was added (3.8 μl), and samples loaded onto a 5% non-denaturing acrylamide gel (acrylamide/bisacrylamide 30 : 1). Electrophoresis was carried out in 1 × TBE at 10 V cm^{-1}. Conditions were similar to those described previously[5,35–37]. *b*, Conditions similar to *a*, but probe (300,000 c.p.m.), 8 μl extract, 6 μg poly[d(I–C)] and 20 mM KCl in a final volume of 21 μl was used. After binding DNase I was added to 50 μg ml^{-1} and digestion was allowed to proceed for 90 s before loading buffer was added. DNA was eluted from the gel[34], resuspended and loaded onto a 6% polyacrylamide–urea gel.

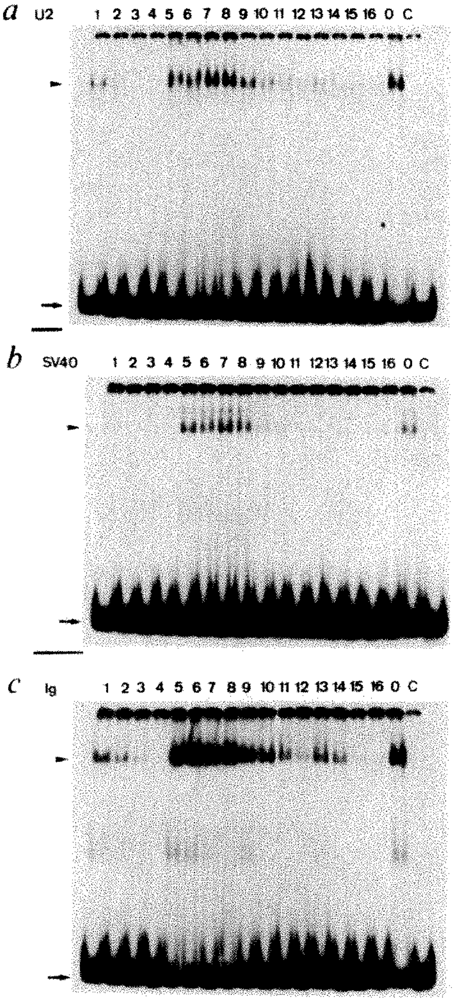
a
U2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 0 C
b
SV40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 0 C
c
Ig
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 0 C

Fig. 3 The three enhancer regions contain binding sites for the same factor. Four double-stranded 40-mer oligonucleotides were synthesized, one corresponding to the U2 DSE region (from position 288–249 (Fig. 1*b*), one to part of the SV40 enhancer (152/224–113/185), one to part of the IG enhancer (567–528) and another control oligonucleotide which did not contain an octamer motif homology. These were then used to compete with one another for binding of factors present in a HeLa cell nuclear extract. *a*, U2 DSE oligonucleotide (3,000 c.p.m., 2 pg) was used in the assay. The major retarded complex is indicated with an arrowhead. Lane C, naked DNA control; lane O, no competitor; lanes 1–4, 50, 100, 200 and 500-fold molar excess of unlabelled U2 DSE oligonucleotide were added as competitor; lanes 5–8 : 50, 100, 200 and 500-fold molar excess of unlabelled control oligonucleotide were added as competitor; lanes 9–12 : 50, 100, 200 and 500-fold molar excess of the SV40 oligonucleotide were added as competitor; lanes 13–16 : 50, 100, 200 and 500-fold molar excess of the IG oligonculeotide were added as competitor. *b*, Analogous to *a*, but with the 5′ end-labelled SV40 oligonucleotide as probe. All competitor concentrations halved with respect to *a*. *c*, Analogous to *a*, but with the 5′ end-labelled IG oligonucleotide as probe. Competitor concentrations as in *b*.
Methods. DNA fragment retardation assays were carried out as described in Fig. 2, except that a HeLa extract was used which had been fractionated on DEAE Sephadex-A25 (see Fig. 4), then bound to heparin-Sepharose in 100 mM KCl and eluted in a step of 500 mM KCl to enrich for the octamer motif binding factor. Competitor and labelled oligonucleotides were added simultaneously following preincubation of the extract with poly[d(I–C)] as in Fig. 2.

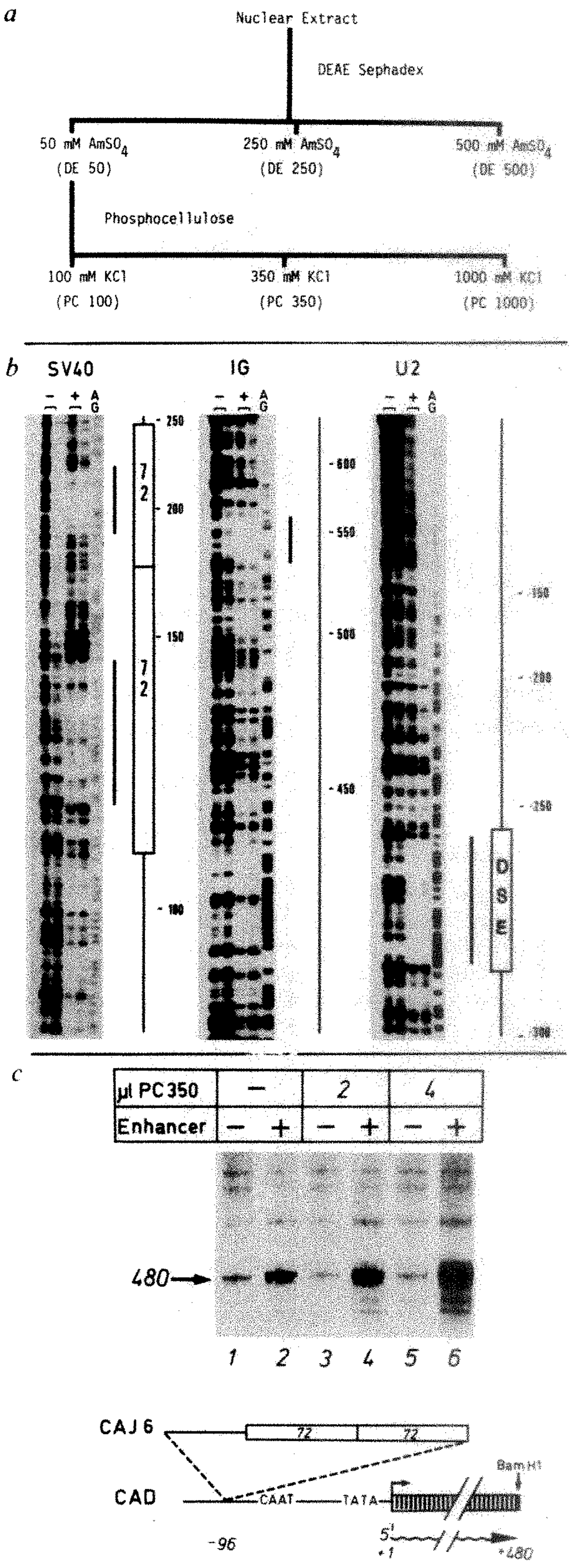

Fig. 4 Partial purification of the octamer binding factor. *a*, Fractionation scheme; *b*, DNase I footprinting assay with fraction PC 350 on SV40, IG and U2 fragments. DNA and footprinting conditions were the same as described in Fig. 1 legend except that 30 µg ml^{-1} of poly[d(I-C)] were used as unspecific competitor. Concentrations of DNase were (from left to right in each panel): 12 and 50 µg ml^{-1} for the control lanes (–) and 50 and 100 µg ml^{-1} for the footprinting lanes (+). For each reaction 10 µl PC 350 were used. Major areas protected against nuclease are indicated by solid lines. Next to each experiment an A+G-specific sequencing lane was run. DSE, U2 distal sequence element. *c*, Stimulation of β-globin transcription *in vitro* by the PC 350 fraction. In plasmid CAJ6 the SV40 enhancer from position 100 to 296 is inserted 96 bp upstream of the β-globin cap-site in its wild-type orientation[7]. The templates were cleaved at a *Bam*HI site in the coding region as shown in the lower part of the figure. The expected length of the run-off transcript is 480 nucleotides. Transcriptions were done in the absence or presence of PC 350 as indicated.

Methods. *a*, HeLa nuclear extract prepared by the standard protocol was dialysed against DE-buffer (20 mM HEPES-KOH (*p*H 7.9), 50 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 20% glycerol) and loaded onto a DEAE-Sephadex A-25 column. The column was washed with loading buffer and eluted with DE-250 and DE-500 buffer (DE-buffer with 250 and 500 mM $(NH_4)_2SO_4$, respectively). Proteins in the flow-through were precipitated with $(NH_4)_2SO_4$, 0.38 g ml^{-1}. The protein pellet was taken up in one tenth the original volume of PC-loading buffer (20 mM HEPES-KOH. (*p*H 7.9), 100 mM KCl, 0.5 mM DTT, 20% glycerol) and dialysed against this buffer. This material was passed onto a phosphocellulose column (Whatman P11), washed and eluted with PC 350 and PC 1000-buffers (PC-loading buffer with 350 mM and 1 M KCl, respectively). Flow-through and step-fractions were dialysed against the standard extract buffer D* (20 mM HEPES-KOH (*p*H 7.9), 20 mM KCl, 2 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 20% glycerol. Protein concentrations: PC 100, 0.72 mg ml^{-1}; PC 350, 0.92 mg ml^{-1}; PC 1000, 0.25 mg ml^{-1}. *b*, Transcription reactions contained (20 µl) 4 µl HeLa nuclear extract, 0–4 µl PC 350, 1 µg truncated DNA template, 185 kBq [α-^{32}P]UTP (15 TBq mmol^{-1}); 5 mM creatine phosphate; 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 10 µM UTP, 20 mM KCl, 4 mM $MgCl_2$, 4 mM spermidine, 10 mM HEPES-KOH (*p*H 7.9), 0.1 mM EDTA, 0.25 mM dithiothreitol, 10% glycerol. The samples were incubated at 30 °C for 60 min and then extracted with phenol/chloroform/isoamylalcohol (25:24:1). The RNAs were precipitated with 2.5 M ammonium acetate and 2.5 vols ethanol, separated on a 6% acrylamide-urea sequencing gel, and detected by autoradiography. Only the part of the autoradiograph containing the specific run-off RNA band (arrow) is shown.

(IgNF-A) specific for the octamer motif in the promoter of an immunoglobulin κ light-chain gene whose binding could be competed by DNA fragments from the IG heavy-chain enhancer. It is therefore likely that IgNF-A and the octamer-binding factor are identical, although the IgNF-A footprint seems to be smaller than the protected regions we detect on several octamer motif regions.

Preliminary results from comparative footprinting experiments (in collaboration with G. J. M. Pruijn, W. van Driel and P. van der Vliet) indicate that nuclear factor III, a protein isolated from HeLa cell nuclei which is required for adenovirus DNA replication in an *in vitro* system[31], recognizes the same DNA sequence as the octamer-binding factor described here.

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Note added in proof: Recent experiments[38] have shown that restriction fragments from the promoters of human U1 snRNA, histone H2B and an immunoglobulin κ-chain gene, all of which contain an octamer motif, compete for the binding of a single factor in HeLa cell nuclear extracts. The octamer motif is required for maximal *in vitro* transcription of the histone H2B gene[39].

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Expression of a polypeptide containing a dipeptide repeat is confined to the insect stage of *Trypanosoma brucei*

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The protozoan parasite *Trypanosoma brucei* is transmitted between mammalian hosts by the tsetse fly (*Glossina* spp.). Trypanosomes ingested by the fly undergo a number of changes in the insect midgut during differentiation to procyclic forms. These include the loss of the variant specific glycoprotein (VSG) coat[1] and the appearance of a common set of procyclic surface antigens[2]. In order to investigate genes other than VSG genes which are expressed only at certain stages of the life cycle, the first cDNA specific to procyclic culture form trypanosomes (equivalent to the stage found in the insect midgut) has been characterized. The encoded polypeptide shows several characteristics of membrane proteins, but its most striking feature is the presence of a repetitive amino-acid sequence in which there are 22 tandem repeats of the dipeptide -Glu-Pro-. Related genes are also found in other trypanosome species and in leishmania. This gene shows many similarities to a number of surface antigen genes described in malaria[3] and, more recently, *Trypanosoma cruzi*[4]. This is the first example of a repetitive sequence in a parasite protein which is present only in the insect vector, and which therefore cannot be implicated in the mammalian host immune response[4-6].

A complementary DNA library was constructed from messenger RNA purified from procyclic culture forms of *T.b. brucei* 427.01 (ref. 7). The library was screened with single-stranded cDNAs synthesized from procyclic and bloodstream mRNAs. Of the plaques that hybridized differentially to these probes, the majority showed only quantitative differences; relatively few displayed absolute stage specificity. This is consistent with the limited number of stage-specific polypeptides among *in vitro* translation products (data not shown). This approach contrasts with methods previously used to characterize stage-regulated gene expression in trypanosomes, which have employed heterologous probes such as *Drosophila* heat shock genes[8]. The first stage-specific clone to be characterized (pPRO2001) hybridized to an mRNA of ~850 bases found in procyclic, but not bloodstream form, trypanosomes (Fig. 1*a*). Even on very long exposure of the autoradiograph there was

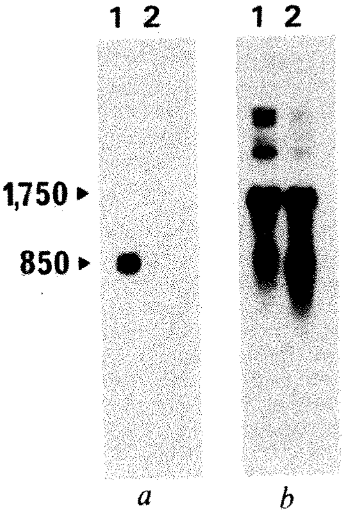

Fig. 1 Northern blot analyses of *T.b. brucei* procyclic culture form and bloodstream form RNAs from the MITaR serodeme[7]. Total RNA was glyoxalated[25] then separated on a 1.3% agarose gel, followed by transfer to nitrocellulose. Lane 1, 5 μg procyclic culture form RNA (427.01); lane 2, 5 μg bloodstream form RNA (MITaT 1.6). The blot was probed first with (*a*) nick-translated ^{32}P-labelled pPRO2001 (ref. 26), then reprobed with (*b*) pPRO301. Size markers in base pairs.

Methods. RNA was isolated from bloodstream and procyclic trypanosomes using the phenol-SDS method[27]. Poly(A) RNA was selected by oligo(dT) chromatography[28]. Double stranded cDNA was synthesized by the strand replacement method[29], prepared for vector ligation using *Eco*RI linkers[30], and inserted into the *Eco*RI site of λNM1149 and a portion of the unamplified library plated on *E. coli* NM514[30]. The screening was performed by plaque hybridization[31] using ^{32}P-labelled single-stranded cDNA synthesized by AMV reverse transcriptase using poly(A) RNA from either bloodstream form or procyclic trypanosomes as template and random hexadeoxynucleotides as primers[32]. Plaques that hybridized with procyclic but not bloodstream cDNA were purified. DNA was isolated from one of these[33] and the cDNA insert subcloned into the *Eco*RI site of pUC8[34] to produce pPRO2001. A second clone, pPRO301, which did not show stage specificity, was isolated from the same library for use as a positive control.

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                                            M  A
TGTACTATATTGACTTCAATTACACCAAAAAGTAAAATTCACAATGGCAC
         10        20        30        40        50

P  R  S  L  Y  L  L  A  V  L  L  F  S  A  N  L  F
CTCGTTCCCTTTATCTGCTCGCTGTTCTTCTGTTCAGCGCGAACCTCTTC
         60        70        80        90       100

 A  G  V  G  F  A  A  A  A  E  G  P  E  D  K  G  L
GCTGGCGTGGGATTTGCCGCAGCCGCTGAAGGACCAGAAGACAAGGGTCT
        110       120       130       140       150

  T  K  G  G  K  G  K  G  G  K  G  T  K  V  S  D
TACTAAGGGAGGCAAAGGCAAAGGCGGGAAGGGAACCAAGGTCAGCGACG
        160       170       180       190       200
         *                    ━━━━━━━━━━━━━━━━━━━━
D  D  T  N  G  T  D  P  D  P  E  P  E  P  E  P  E
ACGATACCAATGGCACTGACCCCGACCCCGAACCTGAACCGGAACCGGAA
        210       220       230       240       250
━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━
 P  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E  P
CCTGAACCTGAACCTGAACCTGAACCCGAACCCGAACCTGAACCTGAACC
        260       270       280       290       300
━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━
  E  P  E  P  E  P  E  P  E  P  E  P  E  P  E  P
TGAACCTGAACCTGAACCAGAGCCAGAACCTGAACCTGAACCCGAACCTG
        310       320       330       340       350
━━━━━━━━━━
E  P  E  P  G  A  A  T  L  K  S  V  A  L  P  F  A
AACCTGAACCTGGTGCTGCAACGCTGAAGTCTGTTGCGCTTCCGTTCGCA
        360       370       380       390       400

 I  A  A  V  G  L  V  A  A  F
ATTGCGGCCGTTGGTCTCGTTGCCGCATTCTAACCTGATGTAAAGAGAAA
        410       420       430       440       450

TCTTTTTGGAGGGTCAAAACTTTTTAACAAGGGTGACCCCAATTGTTTAT
        460       470       480       490       500

TTAACCCTTCCTTTGGCGCGATCCGCTTCATGGCGTTCCTTTGTGACAGG
        510       520       530       540       550

ACACTGACACGCATAATATTTTTTTCAACCCATTTTTTTGAGTTCTATTT
        560       570       580       590       600

TTTGTTTTATGTCTTGGAACCGTCGGCGGTAGCACGCAGCCCTGTAGACT
        610       620       630       640       650

TCCTGCGTGTGCCTGTCAATGTGTTCCTTGGCTCTTTTCAAACGGCTTCT
        660       670       680       690       700

TTTATCTTACCGTTCATATGGTTTTGTCAAAAAAAAAAAAAAAAA
        710       720       730       740
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Fig. 2 Nucleotide sequence of the cDNA insert from pPRO2001 and the inferred amino-acid sequence of the encoded polypeptide. The entire cDNA insert from pPRO2001 was subcloned into the *Eco*RI site of M13mp9[35]. Further subclones were generated by treating *Eco*RI-digested pPRO2001 with *Bal*31 exonuclease, followed by ligation into the *Sma*I site of M13mp9. The nucleotide sequence of the cDNA was determined from these clones by the dideoxy chain termination method[36]. Line, dipeptide repeat region; *, potential glycosylation site.

no evidence for any homologous transcript in the bloodstream form, whereas reprobing the same blot with pPRO301, a clone which does not show stage specificity, demonstrated many hybridizing transcripts in both tracks (Fig. 1*b*).

The first 12 bases of the sequence of the cDNA insert from pPRO2001 (Fig. 2) are exactly homologous to the 3′ end of the 35-base mini-exon sequence which is believed to occur at the 5′ end of most, if not all, trypanosome mRNAs[9,10]; at the other end is the 3′ poly(A) tail. Allowing for the poly(A) tail, the length of the cDNA clone is consistent with that of the hybridizing mRNA (Fig. 1). These observations indicate that the cDNA covers the entire coding sequence. The central portion of the cDNA contains the hexanucleotide ($\mathrm{GA^{A}_{G}CCN}$) repeated 22 times. At the third position of this hexanucleotide adenosine occurs in all but one repeat (Fig. 2). In common with some other trypanosome mRNAs there is a long 3′ untranslated region[11] and no obvious eukaryotic poly(A) addition sequence[12]. The open reading frame shown in Fig. 2 starts with the first ATG triplet and the bases surrounding this codon fit the consensus sequence for most initiation codons[13]. There are two smaller open reading frames of 165 and 198 bases starting with an ATG codon; however the initiation codons are more than 200 bases from the 5′ end of the mRNA and the adjacent sequences do not fit the initiation consensus sequence. The predicted polypeptide (129 amino acids) shows some features of an extrinsic membrane protein. There is an apparent signal sequence[14], a hydrophobic region at the extreme C terminus which may serve as a membrane anchor[15] and a glycosylation site[16] at Asn 56. Some variation occurs in the hexanucleotide repeat, but only in the third base of any codon, which does not alter the encoded amino acid; consequently there is a tandem array of 22 repeats of the dipeptide -Glu-Pro-. In addition, from residue 38 to 47, where there is a tandem repeat of the pentapeptide -Lys-Gly-Gly-Lys-Gly-.

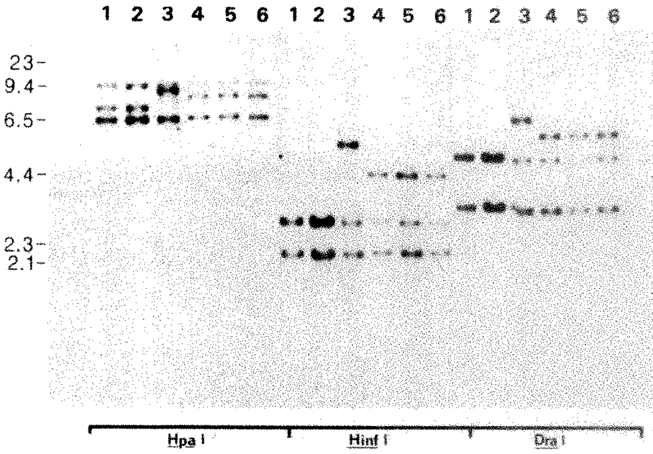

Fig. 3 Southern blot analysis of *T. b. brucei* DNA from the MITaR 1 and ILTaR 1 serodemes. Genomic DNA (1 μg) was digested with *Hpa*I, *Hin*fI or *Dra*I, fractionated on a 0.8% agarose gel and transferred to nitrocellulose[37]. The probe was ^{32}P-labelled pPRO2001[26]. The blot was washed with a solution of 0.1 × SSC, 0.1% SDS at 60 °C for 1 h before autoradiography for 16 h. Lane 1, 427.01 procyclic; lane 2, MITat 1.2 bloodstream form; lane 3, ILTat 1.21 (MIAG 201) procyclic; lane 4, ILTat 1.21 (MIAG 201) bloodsteam form; lane 5, ILTat 1.21 (MIAG 203) bloodstream form; lane 6, ILTat1.25 (MIAG 211) bloodstream form. The 427.01 procyclics were derived from MITat 1.2 bloodstream form trypanosomes (R. F. LePage, personal communication). Size markers, kb.

The gene(s) encoding this procyclic form specific polypeptide show restriction site polymorphism (Fig. 3). The data suggest that one copy of the gene has undergone rearrangement on at least two occasions. The insensitivity of this copy to *Bal*31 exonuclease digestion of genomic DNA shows that the variation in size of the restriction fragment is not due to proximity to a trypanosome telomere[17,18] (data not shown). In addition, all copies of the gene were found on large chromosomes when resolved by pulsed field gel electrophoresis, whereas telomeric VSG genes are also frequently found on intermediate and minichromosomes[19]. In the ILTaR 1 serodeme[20], a Kenyan isolate, no evidence of rearrangement was seen in four bloodstream-

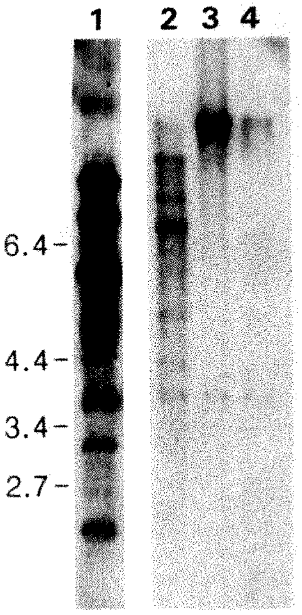

Fig. 4 Southern blot analysis of genes related to pPRO2001 in other Kinetoplastida. Genomic DNA ($1\ \mu g$) was digested with *Pst*I, size fractionated on a 0.8% agarose gel and transferred to nitrocellulose[37]. After hybridization with nick-translated pPRO2001[26] the blot was washed with a solution of 0.1 × SSC, 0.1% SDS at 60 °C for 1 h before autoradiography for 4 d. Lane 1, *T.b. brucei* (427.01); lane 2, *T. congolense* (ILNat 2.1); lane 3, *T. vivax* (1223); lane 4, *L.m. amazonensis* (PH8). Size markers, kb.

form variants that had been cloned by syringe passage (Fig. 3). Interestingly, in a procyclic culture derived from one of these bloodstream clones, polymorphism in one fragment was observed with several restriction enzymes. The serodeme MITaR 1, isolated in Uganda[7], shows a polymorphism which distinguishes it from the ILTaR 1 serodeme (Fig. 3), although no rearrangement was found between a pair of bloodstream and procyclic forms within this serodeme. Certain regions of the trypanosome genome are inherently less stable than others. Both VSG genes and their expression-site associated genes[21] are prone to rearrangement, whereas tubulin genes and various glycolytic enzyme genes are much more stable[22]. The genes encoding the procyclic form specific polypeptide seem to belong to the former class.

Sequences showing a high degree of homology to these genes are also present in other members of the order Kinetoplastida. They can be detected in *Trypanosoma congolense*, *T. vivax* and *Leishmania mexicana amazonensis* (Fig. 4), although the reduced intensity of hybridization suggests that the genes are not exact copies of those in *T. brucei*. Most characterized polypeptides containing tandem repeats occur in protozoa, although there are exceptions such as the glue proteins of *Drosophila*[23,24]. Those identified in *Plasmodium* and *T. cruzi* are antigens which evoke an immune response during the course of infection[3,4]. The only antigens of *T. brucei* which have been well characterized are VSGs and these do not contain any amino-acid repeats. It is significant that these are the surface antigens expressed by trypanosomes which have to contend with the mammalian immune system, whereas the procyclic form specific polypeptide containing amino-acid repeats is expressed by a life cycle stage which is confined to the insect. This finding argues against tandem amino-acid repeats having originally evolved in parasitic protozoa to provide protection exclusively in the mammalian host.

We thank Judith Creighton for bloodstream form trypanosomes, Alistair Ross and Jerry Wells for procyclic forms, Phelix Majiwa for gifts of *T. congolense* and *T. vivax* DNA, Lorna Gibson for *L. m. amazonensis* DNA, Dick Williams, Dirk Dobbelaere and Amanda Gill for criticizing the manuscript, and Josie McConnell and Angelika Ehrfeld for advice and encouragment.

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Transgenic rye plants obtained by injecting DNA into young floral tillers

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The most successful method currently used to produce transgenic plants is based on the ability of *Argobacterium tumefaciens* to infect a number of higher plant species and transfer a defined DNA fragment (T-DNA) to the genome of the infected cells[1]. This procedure cannot be used with the agriculturally important cereals, although preliminary data have suggested[2] infection of maize seedlings by *A. tumefaciens* strains. Purified exogenous DNA can be taken up, integrated and expressed in cells of a variety of plant species including some cereals following direct gene transfer into isolated protoplasts[3–7]. However, although it is possible to grow isolated cereal protoplast into unorganized tissue (calli)[5,7,8], only rice protoplasts have so far been shown to regenerate mature plants[9–11]. Here we report an alternative approach to transformation of cereal plants which does not involve tissue culture techniques.

Previous work[12,13] on the development of the male germ line of rye (*Secale cereale* L.) has shown that at 14 days before the first meiotic metaphase the archesporial cells are highly sensitive

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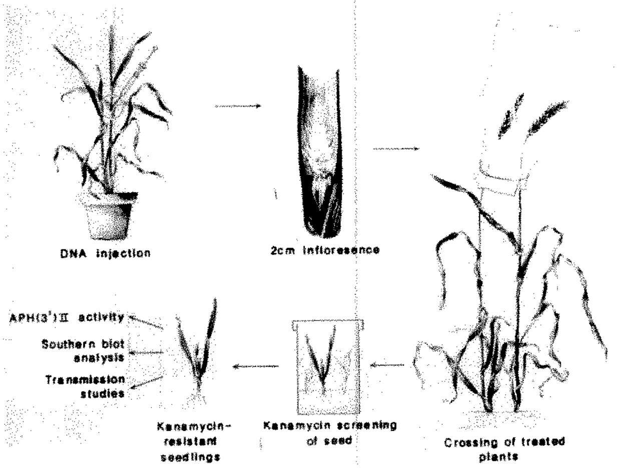

Fig. 1 Scheme of the transformation and screening strategy. Aliquots of an aqueous solution (100 μg ml^{-1}) of pLGVneo1103 were injected above each tiller node until several drops of the solution came out from the top of the tiller (usually a total injected volume of 300 μl). The plants were injected about 14 days before meiosis using a tuberculin syringe (25G 5/8). This stage was recognized by the morphology of the developing tiller. When young tillers have 5 leaves and the flag leaf reaches one third to one half of its final size, they contain, at approximately the level of the third leaf, a young influorescence of about 2 cm which is suitable for injection. After injection, the floral tillers were allowed to grow to maturity. Seeds from these injected tillers were obtained by cross-pollination with other injected tillers. This cross-pollination was necessary because JNK rye is strongly self-incompatible.

to caffeine and colchicine injected into the developing floral tillers. We considered that at this stage the archesporial cells might also be permeable to other molecules, such as DNA. Therefore we have injected DNA carrying a dominant selectable marker gene into rye plants, under precisely the same conditions as those leading to meiotic abnormalities after caffeine or colchicine injections.

The plasmid pLGVneo1103 (Fig. 3*b*) carrying the aminoglycoside phosphotransferase II gene (APH(3')II) under the control of the nopaline synthase (NOS) promoter (L. Herrera-Estrella, personal communication; ref. 4) was injected into developing floral tillers of the diploid rye cultivar JNK. The plants were injected 14 days before meiosis (Fig. 1). JNK rye expresses a high level of self-incompatibility, therefore, seeds were obtained by pair-wise crossing of injected tillers from different plants. The seeds set on the injected tillers were screened for kanamycin resistance. Glass containers (3l) were filled under aseptic conditions with about 400 ml of Knop nutritive solution[14,15], supplemented with 10 μg ml^{-1} of kanamycin sulphate (Sigma). A plastic net was positioned in these containers such that seeds deposited on it would lie just above, and in contact with, the nutritive solution. A maximum of 10 surface sterilized seeds were tested in each container and grown in a culture room at 26 °C at approximately 2,000 lx for 16 h per day. Control seeds, from untreated rye plants, were germinated under the same conditions with and without kanamycin. In the presence of kanamycin, control seedlings bleached and were almost totally white after 10 days. From 3,023 seeds derived by cross-pollinating 98 plants injected with pLGVneo1103, which were tested with this procedure, seven seedlings remained green after 10 days growth on kanamycin-containing medium. These apparently kanamycin resistant seedlings were assayed for the presence of APH(3')II enzymatic activity[16,17]. Two of these seven 'kanamycin resistant' plants, each resulting from an independent injection experiment, showed APH(3')II activity (Fig. 2, lanes *f, h*). No APH(3')II activity was detected in about one hundred control rye seedlings or in 30 bleached kanamycin-sensitive seedlings derived from injected tillers.

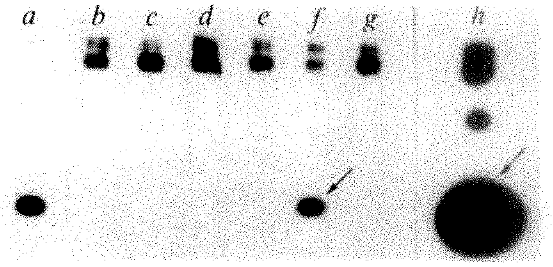

Fig. 2 Detection of APH(3')II activity in plant extracts. Leaf material (~100 mg) was crushed in an Eppendorf tube with 70 μl of extraction buffer[17]. Protein extracts (50 μl) were electrophoresed onto a 10% polyacrylamide gel, which was incubated with kanamycin and [γ-^{32}P]ATP and blotted onto Whatman phosphocellulose paper p81[17]. *a*, APH(3')II-transformed tobacco (positive control); *b*, Untreated JNK rye (negative control); *c–e*, untransformed kanamycin-sensitive seedlings from injected plants; *f*, kanamycin-resistant seedlings; *g*, apparently kanamycin resistant seedling showing no APH(3')II activity; *h*, APH(3')II activity detected in a second kanamycin-resistant seedling resulting from an independent experiment. Arrow, ^{32}P-labelled band of kanamycin that comigrates with one resulting from APH(3')II activity in a transformed tobacco plant provided by Dr P. Czernilofsky. The bands present in all the rye extracts have been observed in variable form for several plant species and are thought to represent protein kinases[5,7,17].

To confirm that the observed APH(3')II activity was due to the presence of the introduced pLGVneo1103 DNA in the plant genome, DNA/DNA hybridizations[18] in which DNA isolated from the APH(3')II-positive plants, as well as from one APH(3')II-negative but apparently kanamycin resistant plant, were performed using a cloned 1.6 kilobase (kb) *Pst*I fragment of pLGVneo1103 internal to the APH(3')II gene as a specific probe (Fig. 3*b*). No hybridization was found in DNA isolated from control rye plants (lane *d*) while in the two APH(3')II-positive plants a 3 kb hybridizing band, corresponding to the *Eco*RI-*Hin*dIII fragment of pLGVneo1103, was detected (lanes *f, h*). Two additional bands, of 2.6 kb and 1.8 kb, were found in the DNA of one of the transformed plants (lane *f*) probably representing rearranged copies of the introduced gene, a common feature in gene transformation by direct DNA uptake[4,7,19]. The DNA from one of the five 'kanamycin resistant' plants which did not show any APH(3')II activity did not contain any fragment hybridizing to the APH(3')II probe (lane *i*). This indicates that plants can remain green throughout the screening procedure without having incorporated the selectable marker although it reinforces the correlation between APH(3')II activity and actual DNA incorporation. The 'leaky' character of the kanamycin selection observed in JNK rye might be the consequence of the genotypic heterogeneity of this cultivar resulting from its cross-pollinating mating behaviour. Genomic DNAs digested separately with *Eco*RI or with *Hin*dIII, each cutting at a unique site in pLGVneo1103, showed several hybridizing bands (data not shown). This result is as expected if the introduced DNA is integrated in the genomic DNA at more than one site or as more than one copy, and the hybridizing bands larger than the 7.15 kb of pLGVneo1103 can also be explained in this way.

These results show that it is possible to introduce new genetic information into germ cells of cereals and to recover seeds capable of growing into normal plants in which the foreign gene is present and expressed. In a repeat experiment, 37 JNK plants were similarly injected with DNA of another plasmid carrying the same APH(3')II gene. Out of about 1,000 derived seeds, three were 'kanamycin resistant' and one of these showed APH(3')II activity. The occurrence of this third APH(3')II positive plant indicates the reliability of the transformation procedure. The method is based on delivery of DNA, carrying a

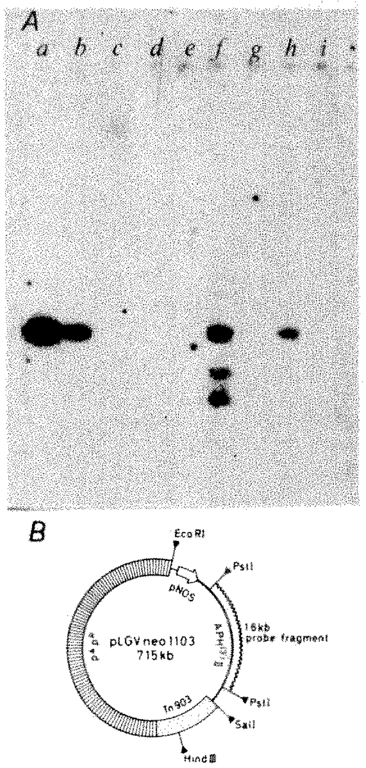

Fig. 3 DNA analysis of the APH(3')II positive plants. *A*, DNA was isolated from leaf material[23] and double digested with *Eco*RI and *Hin*dIII. Approx. 5 μg of digested DNA was analysed by electrophoresis in 0.8% agarose gel and blotted[18]. The probe was the 1.6 kb *Pst*I internal fragment of the APH(3')II gene. Lanes *a* and *b*, pLGVneo1103 DNA digested with *Eco*RI and *Hin*dIII (lane *a* contains an amount of pGLVneo1103 DNA approximately equivalent to 10 copies of plasmid DNA per haploid rye genome and lane *b* contains two copies equivalent); lane *c*, *Hin*dIII-digested λDNA, used for size marking of DNA fragments. Lane *d*, control JNK rye DNA digested with *Eco*RI and *Hin*dIII; lane *f*, DNA from APH(3')II positive plant 1; lanes *e*, *g*, blank. The arrow shows the expected 3 kb band comigrating with the diagnostic 3 kb *Eco-Hind* pLGVneo1103 fragment. Lane *h*, DNA from APH(3')II-positive plant 2. The arrow shows the expected 3 kb band. Lane *i*, DNA from apparently kanamycin resistant but APH(3')II-negative plant. *B*, Diagram of pLGVneo1103 showing the *Eco*RI and *Hin*dIII sites and the 1.6 kb *Pst*I fragment used as a probe.

selectable marker gene into young floral tillers. We postulate that the DNA is subsequently transported by the plant vascular system to the germ cells which can take it up only if they are in a 'competent' stage. In our rye cultivar this competent stage occurs two weeks before meiosis and corresponds cytologically to the interphase before the penultimate premeiotic mitosis in male tissue[13], when male germ cells are not surrounded by a callose wall[21]. (At this time female cells are in an equivalent developmental stage[20]). These cells undergo several changes in response to commitment to meiosis[22], perhaps the most important in relation to DNA uptake being the establishment of cytoplasmic channels connecting the archesporial cells. It is conceivable that DNA molecules could pass through these structures[22]. At present we do not have any evidence to indicate whether DNA uptake occurred in the male or the female germ line or in both. The membrane characteristics of the male cells at the time of injection make them very likely candidates. Further experiments will test this hypothesis.

We are confident that this simple transformation procedure can be extended to other cereals, which have a premeiotic development equivalent to that of rye and for which, no other transformation system leading to plants is currently available. Until now it has been generally accepted[8] that transformation and regeneration of isolated protoplasts of cereals were necessary steps to obtain transgenic cereal plants. Our results demonstrate that other strategies are worth exploring.

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Quisqualate receptors are specifically involved in cerebellar synaptic plasticity

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Long-term modification of transmission efficacy at synapses is the cellular basis of memory and learning[1-4]. A special type of synaptic plasticity in the cerebellum was postulated theoretically[5,6], and has since been verified[4,7-9]. Each cerebellar Purkinje cell (PC) receives two distinct excitatory inputs, one from parallel fibres (PFs) and the other from a climbing fibre (CF). When these two types of inputs are conjunctively activated, PF–PC transmission undergoes long-term depression (LTD)[4,7-9]. Accumulated evidence suggests that LTD plays a role in the motor learning processes of the cerebellum[10-12]. At the molecular level, LTD appears to be caused by desensitization of receptor molecules in PC dendrites towards the PF neurotransmitter[4], presumably L-glutamate (Glu) (ref. 12). Glu receptors are heterogeneous and can be divided into several subtypes[13-15]. In this study, we compared the potency of several Glu agonists in inducing LTD and found a highly selective dependency of LTD on the quisqualate(QA)-selective subtype of Glu receptors.

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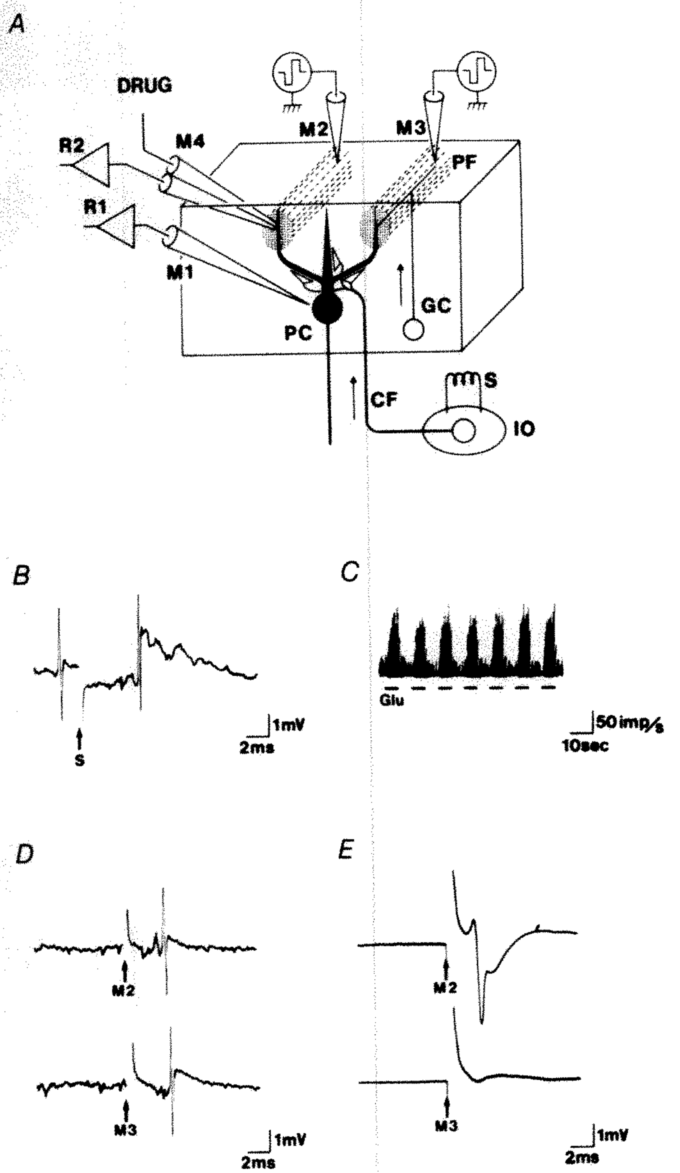

Fig. 1 Experimental system. *A*, Schematic drawing of the cerebellar cortex with recording and stimulation electrodes attached. M1, M2 and M3, glass-coated tungsten microelectrodes. The tip of M1 was exposed for 15 μm, and those of M2 and M3 for 100 μm. M4, double- or triple-barrelled glass microelectrode for application of drugs. The individual barrels of M4 contained the following solutions: L-glutamate (Glu, 0.2 M, *p*H 7.0), L-aspartate (Asp, 0.2 M, *p*H 7.0), quisqualate (QA, 0.01 M in saline, *p*H 6.0), kainate (KA, 0.01 M in saline, *p*H 7.0), *N*-methyl-D-aspartate (NMDA, 0.01 M in saline, *p*H 8.0), 2-amino-5-phosphonovalerate (APV, 0.2 M, *p*H 8.0), kynurenate (KYNA, 0.2 M, *p*H 8.0). R1 and R2, AC amplifiers (R1, band width, 0.08–10,000 Hz, connected to M1; R2, band width, 15–10,000 Hz, connected to the recording barrel of M4). S, bipolar platinum-iridium electrode for stimulation of climbing fibres (CFs); PC, Purkinje cell; GC, granule cell; PF, parallel fibre; CF, climbing fibre; IO, inferior olive. *B*, Extracellular recording from a PC with R1. The arrow marks the moment of IO stimulation. Note that just before the stimulus artifact, there is a spontaneous simple spike. *C*, Stripe chart record of a simple spike discharge from the same PC as *B*. Bin width, 0.5 s. Horizontal bars under the record show the periods of Glu application. *D*, Record from the same PC as *B*, but with M2 and M3 stimulation. *E*, Field potentials evoked with M2 and M3 stimulation recorded with R2. Note that marked field potentials were evoked with M2 stimulation, but not with M3 stimulation. These field potentials consist of a characteristic initial negative spike, n_1, and a succeeding negativity, n_2, representing PF volleys and excitation of cortical cells evoked thereby. *B* and *D* are single sweep records. *E* is the average of 8 sweeps, repeated at a rate of 0.5 s^{-1}.

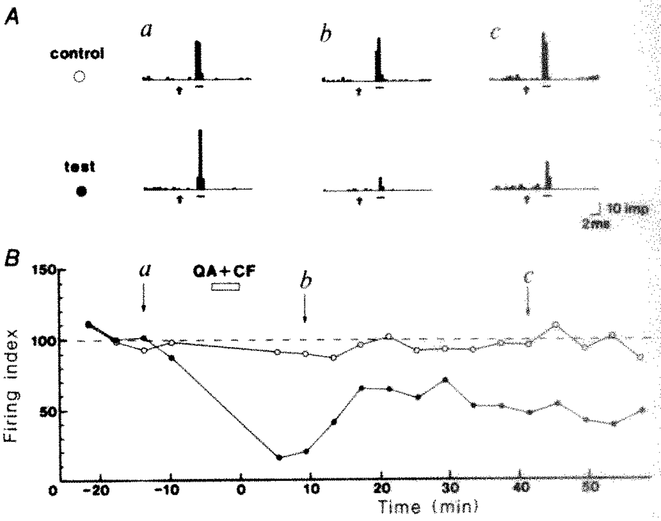

Fig. 2 Depression of PF–PC transmission induced by conjunctive iontophoretic application of QA to PC dendrites with electrical stimulation of CFs. *A*, Peristimulus time histograms of PC responses to stimulation of the control (upper) and the test (lower) PF beams, each constructed during 120 successive stimuli repeated at the rate of 0.5 Hz. Horizontal bars below histograms, stimulus-induced firing of the PC. Bin width, 0.5 ms. Arrows, moments of PF stimulation (*a*) before and (*b*) 9 min and (*c*) 40 min after conjunctive application of QA with 4 nA for 4 min with CF stimulation, as indicated in *B*. *B*, Values of the firing index for stimulation of the control (○) and the test (●) PF beams, relative to the control values prior to the QA–CF conjunction (horizontal open bar). Times *a*, *b* and *c* are as in *A*. Firing index was calculated as the number of evoked spikes (total spike number minus the expected number of spontaneous spikes) during a 1.5 ms period (horizontal bars below histograms in *A*) divided by 120, the number of stimulation trials.

Experiments were performed on the superficial folium of the dorsal paraflocculus of decerebrate rabbits, prepared as described elsewhere[4,7,8]. Figure 1 shows a diagram of the experimental set-up and specimen records. A glass-coated tungsten microelectrode (M1) was placed on a PC which was identified by characteristic CF responses to olivary stimulation (Fig. 1*B*). Two glass-coated tungsten microelectrodes (M2, M3), separated by a distance of 150–200 μm from each other, were inserted into the molecular layer at about 500 μm from the PC so as to activate the PC through separate PF beams (Fig. 1*D*). A drug-containing glass micropipette (M4) was placed on the dendrites of the PC being recorded, identified by responses of the PC to an iontophoretically applied Glu agonist (Fig. 1*C*). Then the position of M4 was adjusted so that it was on one of the PF beams, the test beam, and field potentials evoked with M2 were recorded through M4 (Fig. 1*E*). Stimulation of another PF beam, the control beam with M3 did not evoke any noticeable potentials (Fig. 1*E*). The stimulus strength for each PF beam was 4–15 μA (biphasic constant current pulses, total duration of 0.4 ms) which excited the PC in 40–100% of the stimulation trials. The efficacy of PF–PC transmission was quantified by calculating the firing index[4,8] on peristimulus time histograms constructed from 120 successive trials of PF stimulation (Fig. 2*A*). The two PF beams were tested simultaneously by alternating stimulation, each at a stimulation frequency of 0.5 Hz.

When a PC exhibited stable PF responsiveness for at least 10 min, conjunctive CF stimulation with local application of a Glu agonist was performed in the following manner. While Glu, L-aspartate (Asp), QA or kainate (KA) was ejected from the drug-containing electrode by pulsative iontophoresis (pulse duration: 2–5 s), CFs were stimulated at 2 Hz. The CF stimulation was continued for 5 s and then was interrupted for 5 s. These stimulation trials continued for 4 min. Pulsative iontophoretic

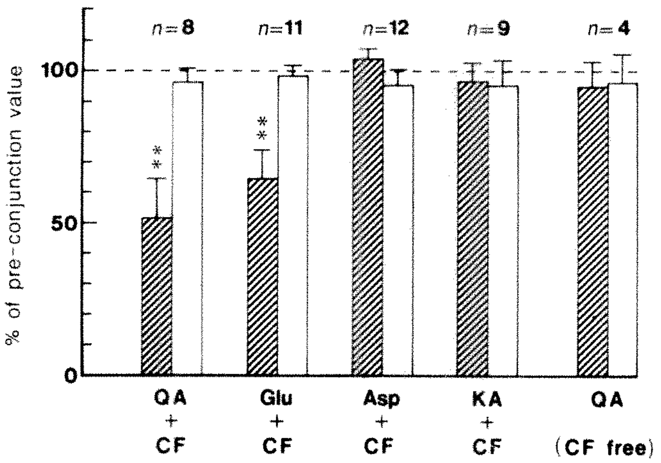

Fig. 3 Relative potency of Glu agonists. Mean percentage reduction of the values of the firing index in stimulation of the test (hatched bars) and the control (open bars) PF beams, at 20-30 min after termination of conjunction of CF stimulation and application of the Glu agonist indicated under the abscissa. Note that in the case of QA (CF free), QA alone was applied in the absence of both spontaneous and stimulus-evoked CF responses. Vertical lines, s.e.m.; *n*, number of cells tested; **, $P<0.01$ for one-sided signed Wilcoxon test for reduction of the values of the firing index relative to its pre-conjunction value.

application of a Glu agonist was synchronized with this alternating CF stimulation, but the onset time and the duration of the drug ejection were varied in order to induce an optimal increase in the simple spike discharge from the PC during the period of CF activation (duration: 5 s for Glu and Asp, 3 s for QA, 2 or 3 s for KA). The intensity of ejection currents was chosen so that the instantaneous frequency of a simple spike discharge from the PC increased to 100-150 s^{-1} (−5 to −30 nA for Glu, Asp and KA, −1 to −15 nA for QA; retention currents, +5 nA). Adoption of this rather complicated procedure was necessary for obtaining constant activation of the PC during conjunction. Otherwise, the PC might be depressed during continuous CF stimulation and drug application. In four experiments, the spontaneous occurrence of complex spikes was reversibly suppressed by local injection of lidocaine (4%, 2-6 μl) through a microsyringe inserted into the region of the contralateral inferior olive (IO). QA was then applied in the absence of CF inputs with ejection current and ejection time equivalent to those used for QA-CF conjunction.

QA-CF conjunction regularly induced a depression of the responsiveness of the PC to stimulation of the test beam (Fig. 2). The depression reached its maximum within 20 min and lasted for more than 50 min. By contrast, there was no noticeable change in the PC responsiveness to stimulation of the control beam, which was located about 150 μm from the QA-ejecting electrode. Similar depression was observed with Glu-CF conjunction, but never with Asp-CF or KA-CF conjunction (Fig. 3). QA is thus the only Glu agonist which effectively depressed PF-PC transmission in a manner similar to Glu. Application of QA in the absence of spontaneous CF activity produced no effect on the PC responsiveness to stimulation of the test beam, as confirmed in four PCs (Fig. 3).

Conjunctive application of any of the four Glu agonists with CF stimulation did not affect the PC responsiveness to stimulation of the control beam. Since the drug-applying electrode was located 150-200 μm from the stimulation electrode for the control beam, the dendritic region of the PC activated by the control beam was presumably free of influences from the drug applied to the test beam region. Furthermore, QA application in the absence of spontaneous CF activity was ineffective in depressing PF-PC transmission. Therefore, it is evident that conjunction of CF activity and QA or Glu application is indispensable for inducing a depression of PF-PC transmission.

Neither Asp nor KA induced a depression of PF-PC transmission in spite of their excitatory effects on PCs. This implies that the effect of Glu and QA in depressing PF-PC transmission does not merely result from depolarization or consequent simple spikes evoked in the PC. Both Asp-selective and KA-selective receptors appear to be present on the PC membrane[16-19], but these receptors are apparently not involved in the depression of PF-PC transmission.

Another Glu agonist, *N*-methyl-D-aspartate (NMDA), was not included in the test described above, because application of NMDA (ejection current: −25 to −40 nA) by pulsative iontophoresis (pulse duration: 6-10 sec) did not activate PCs, as reported previously[16,17]. When applied to the dendritic region of a PC, NMDA usually induced an inhibition of a simple spike discharge, presumably through activation of inhibitory interneurones[20]. In order to examine whether NMDA receptors are responsible for PF-PC transmission, experiments were performed using a selective NMDA antagonist, 2-amino-5-phosphonovalerate (APV), together with a non-selective Glu receptor antagonist, kynurenate (KYNA) (ref. 20). Iontophoretic application of APV (−30 nA for 4 min) significantly blocked the NMDA-induced inhibition (Table 1). However, the same dosage of APV did not antagonize the PC responses to both QA application and PF stimulation (Table 1). By contrast, iontophoretic application of KYNA (−40 nA for 4 min) significantly antagonized both PF-PC transmission and the QA-induced excitation (Table 1). These results indicate that PF-PC transmission is mediated, at least mostly, at non-NMDA receptors.

Table 1 Effects of APV and KYNA on responses of Purkinje cells to NMDA, QA and PF stimulation

	Percentage blockade		
	NMDA*	QA	PF stimulation
APV; −30 nA ($n=8$)	82.2 ± 6.3†	4.9 ± 4.7	5.8 ± 3.4
KYNA; −40 nA ($n=8$)	—	77.4 ± 7.7†	82.3 ± 6.7†

Values are mean ± s.e.m. APV and KYNA were continuously applied (4 min with current shown) onto the dendritic region of the PC.

* NMDA evoked inhibitions on PCs.

† $P<0.01$, one-sided signed Wilcoxon test; before versus after application of either APV or KYNA.

Since the depression of PF-PC transmission induced by QA- and Glu-CF conjunction is similar in time-course and magnitude to that observed after PF-CF conjunction, it follows that iontophoretically applied QA or Glu replaces electrical stimulation of PFs in induction of LTD. The present results thus strongly suggest that QA-selective Glu receptors in PC dendrites play major roles in PF-PC transmission and its LTD. The mechanism of LTD remains a matter of speculation, but Ca^{2+} influx accompanying CF responses seems to play an essential role. This is suggested by the following results. Activation of CFs evokes prolonged plateau potentials in PC dendrites[21], which represent Ca^{2+} influx[22]. When these plateau potentials are suppressed by postsynaptic inhibition of PC dendrites through stellate cells, conjunctive stimulation of PFs with CFs fails to induce LTD (ref. 8). In horizontal cells of goldfish retinae, application of QA alone leads to desensitization of QA receptors[23]. However, this desensitization itself is insufficient to explain the mechanism of LTD, since it recovers quickly after termination of QA application[23]. In order for LTD to occur, sufficient Ca^{2+} inflow into PC dendrites is necessary. It is probable that the QA receptors on PC dendrites undergo long-lasting biochemical modification, such as phosphorylation of receptor protein, under conjoint influences of Ca^{2+} ions from the inside and Glu or QA from the outside.

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Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid

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Cellulose is the most abundant renewable carbon resource on earth and is an indispensable raw material for the wood, paper, and textile industries. A model system to study the mechanism of cellulose biogenesis is the bacterium *Acetobacter xylinum* which produces pure cellulose as an extracellular product. It was from this organism that *in vitro* preparations which possessed high levels of cellulose synthase activity were first obtained in both membranous and soluble forms$^{1-3}$. We recently demonstrated that this activity is subject to a complex multi-component regulatory system^{4,5}, in which the synthase is directly affected by an unusual cyclic nucleotide activator enzymatically formed from GTP, and indirectly by a Ca^{2+}-sensitive phosphodiesterase which degrades the activator. The cellulose synthase activator (CSA) has now been identified as bis-(3′→5′)-cyclic diguanylic acid (┌5′G3′p5′G3′p┐) on the basis of mass spectroscopic data, nuclear magnetic resonance analysis and comparison with chemically synthesized material. We also report here on intermediary steps in the synthesis and degradation of this novel circular dinucleotide, which have been integrated into a model for the regulation of cellulose synthesis.

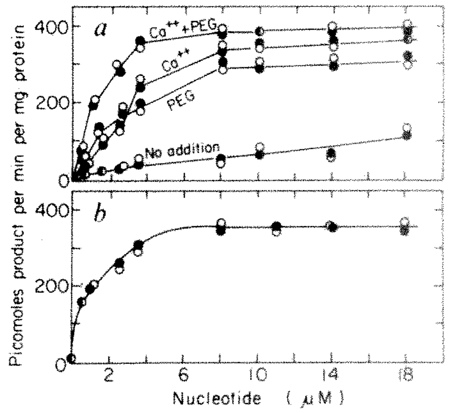

Fig. 1 Effects on activation of cellulose synthase by (●) native CSA and (○) synthetic c-di-GMP of Ca^{2+} and PEG. *a*, Synthase in washed membranes^{1,2,4}. Standard reaction mixtures contained (as shown) 8% PEG 4000, 1 mM CaCl$_2$, 1 mM CaCl$_2$ plus PEG, or no addition. *b*, Digitonin-solubilized synthase^3. Activity as presented was not affected by addition of CaCl$_2$ or PEG. Concentrations of nucleotide were calculated using a molar extinction coefficient of 24,700 at 252 nm, *p*H 7.0.

Methods. CSA was prepared enzymatically from GTP (ref. 5), modified by employing affinity purified diguanylate cyclase. Synthesis of c-di-GMP and 3′-5′ cyclic triguanylic acid was by a published procedure^{12} with slight modification (E. de V., G.A. van der M. and J.H. van B., in preparation). Their structures were unambiguously ascertained by ^{1}H- and ^{31}P-NMR spectroscopy.

A cyclic structure, consisting of two or more riboguanosine residues linked by 2′-5′ or 3′-5′ phosphodiester bonds, was proposed for the CSA^{4,5}. Direct support for a cyclic dimeric structure was obtained by DEAE-Sephadex chromatography^6, which indicates that the compound contains not more than two GMP residues, and by mass spectroscopic analysis (fast atom bombardment in the negative ion mode), which indicates that its relative molecular mass (M_r) is 690, a value corresponding to that of cyclic diguanylic acid. That the CSA is indeed the 3′-5′ isomer of cyclic diguanylic acid, namely bis-(3′→5′)-cyclic diguanylic acid (c-di-GMP), was first indicated by its susceptibility to the 3′-5′ phosphodiester-specific T1 endonuclease^7, and finally established by chemical synthesis of the putative compound. The chemically synthesized material and the enzymatically formed activator are: (1) identical in stimulating cellulose synthase activity under a variety of conditions (Fig. 1); (2) show the same sensitivity and yield identical products when subjected to a variety of chemical and enzymatic treatments^{4,5}; (3) yield identical proton and phosphorus NMR (nuclear magnetic resonance) spectra (Fig. 2); and (4) are indistinguishable by HPLC analysis (Fig. 3*b*). To the best of our knowledge, our findings represent the first case of a cyclic dinucleotide of biological origin observed with a defined physiological function, namely the regulation of cellulose biosynthesis.

The enzyme responsible for the synthesis of the c-di-GMP activator, referred to here as diguanylate cyclase, has been purified 3000-fold from the supernatant of washed membranes. Results of parallel experiments, using either [γ-^{32}P]- or [α-^{32}P]GTP as substrate, indicate that in the diguanylate cyclase reaction, one mole of pyrophosphate (PPi) is released for every mole of GMP residue incorporated from GTP into c-di-GMP (Fig. 3*a*). In both reaction mixtures, an additional ^{32}P-labelled product is formed in small amounts, containing twice as much [α-^{32}P]GTP-derived label as that from [γ-^{32}P]GTP. This material, referred to as 'product C', is devoid of stimulatory activity and apparently is an intermediate in the diguanylate cyclase reaction; when subjected as sole substrate to the action of the purified enzyme, product C is completely converted to c-di-GMP (Fig. 3*b*). Taking together the stoichiometry of the cyclase reaction, the structure of its final product and the pattern

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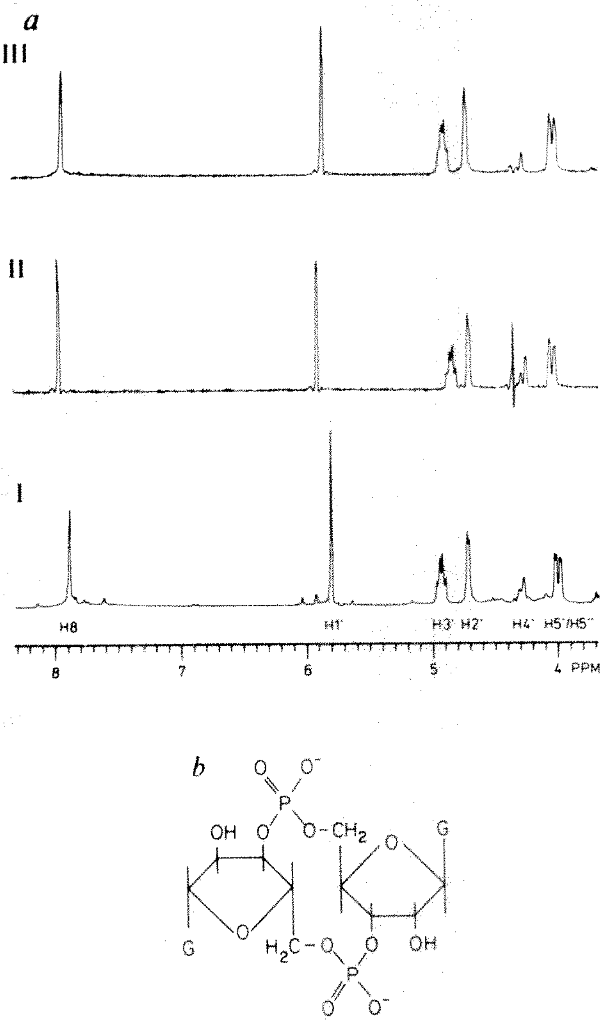

Fig. 2 *a*, NMR spectroscopy of native CSA and synthetic c-di-GMP. ^{1}H-NMR spectra of (I) naturally occurring CSA, (II) synthetic c-di-GMP, (III) equimolar mixture of native CSA and synthetic c-di-GMP, recorded at 50 °C (D_2O, 300 MHz); chemical shifts (δ-values) are given in p.p.m. relative to the internal standard tetramethylammonium chloride (δTMA = 3.19 p.p.m.). The assignments of the low-field ribose protons H1' to H5' are based upon 2D-COSY spectra of the synthetic and natural materials. The H1' protons of synthetic c-di-GMP and natural CSA have a coupling constant ($J_{H1',H2'}$) of 1.8 Hz. Also, ^{31}P-NMR (D_2O; 80.7 MHz) spectroscopy of a mixture containing synthetic c-di-GMP and natural CSA revealed the presence of one resonance at −0.62 p.p.m. relative to the external standard aqueous (85%) H_3PO_4. *b*, Structural formula of bis-(3'→5')-cyclic diguanylic acid (c-di-GMP).

of ^{32}P incorporation from GTP into product C, this presumed intermediate is most likely to be the linear diguanosine-tetraphosphate pppG3'p5'G. This conclusion is borne out by the finding that synthetically prepared pppG3'p5'G is identical to product C with respect to HPLC retention and in its ability to serve as a precursor in the diguanylate cyclase reaction (Fig. 3*b*).

The target enzyme for c-di-GMP action is the membrane-bound cellulose synthase. Activation of this enzyme is highly specific for the dimeric structure, since neither the lower homologue, 3',5' cyclic GMP, nor the higher homologue, 3'-5' cyclic tri-guanylic acid (chemically synthesized, Fig. 1 legend) at concentrations up to 0.5 mM, stimulate the enzyme. In the entire range of c-di-GMP concentrations tested, stimulation of

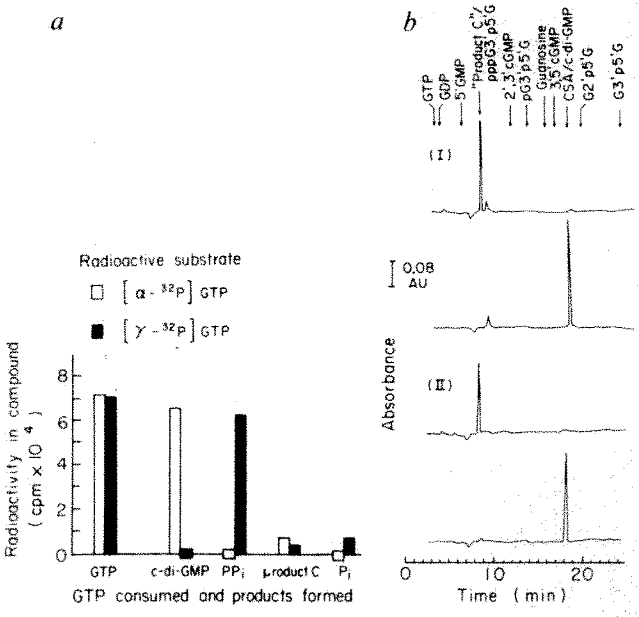

Fig. 3 Characterization of the diguanylate cyclase reaction. *a*, Stoichiometry of the reaction. The values attained for the [α-^{32}P]GTP reaction mixture have been multiplied by 1.2 in the diagram to correct for the higher specific radioactivity in the [γ-^{32}P]GTP reaction mixture. *b*, Conversion of product C and pppG3'p5'G to c-di-GMP. Diguanylate cyclase (0.1 μg protein) was incubated with either (I) 2.1 nmol of synthetic pppG3'p5'G or (II) 1.2 nmol of purified product C and 1 mM $CaCl_2$ in 0.05 ml TME buffer. Presented is the HPLC elution profile of the reaction mixtures at zero time (top trace in each part) and 20 min (bottom trace).

Methods. Diguanylate cyclase was purified from the supernatant of washed membranes by precipitation in 10% PEG, followed by DEAE-cellulose chromatography, employing a linear gradient of 0.05–0.3 M KCl in 60 mM Tris-HCl, *p*H 7.5, containing 10 mM $MgCl_2$ and 1 mM EDTA (TME buffer), and finally by affinity chromatography[4], modified by including 1 mM dithiothreitol (DTT) and 10% glycerol in the GTP eluate. The GTP concentration in the enzyme-containing GTP-eluted fractions was reduced to 30 nM by vacuum dialysis. In *a*, reaction mixtures, containing 0.2 mM of either [α-^{32}P]GTP (100,000 c.p.m. nmol^{-1}) or [γ-^{32}P]GTP (120,000 c.p.m. nmol^{-1}), 3 mM sodium pyrophosphate (PPi) (to minimize degradation of the labelled PPi released), 1 mM $CaCl_2$, and vacuum dialysed enzyme (0.1 μg protein) in 0.05 ml TME buffer, were incubated for 15 min at 30 °C. Reaction was terminated by applying 10 μl of each reaction mixture to polyethyleneimine-cellulose plates which were developed in 5.5 M $(NH_4)_2SO_4$, *p*H 3.5, for determining c-di-GMP (R_F 0.03) and product C (R_F 0.14), and in 1.5 M KH_2PO_4, *p*H 3.65, for determining GTP (R_F 0.38); Pi (R_F 0.80); and PPi (R_F 0.66). Labelled spots, detected by autoradiography, were extracted and counted. In *b*, product C was isolated from scaled-up diguanylate cyclase reaction mixtures and purified by HPLC fractionation; pppG3'p5'G was synthesized as reported[13]. The identity and homogeneity of the product were corroborated by ^{1}H and ^{31}P-NMR spectroscopy. HPLC was performed on a 250 × 4 mm RP-18 column (Merck) at 40 °C, detection at 252 nm, on a Merck-Hitachi liquid chromatograph. Runs were carried out in 0.15 M NaH_2PO_4, *p*H 5.2, at 1 ml min^{-1}, using a multi-step gradient of acetonitrile (steps are isocratic, unless otherwise stated); 1 min (0–2% linear gradient), 1–12 minutes (2%); 12–15 minutes (2–6% linear gradient), 15–20 minutes (6%), 20–25 minutes (6–0% linear gradient). Preparation of [terminal 5'-^{32}P]pG3'p5'G was by 5' phosphorylation of G3'p5'G with [γ-^{32}P]ATP and polynucleotide kinase (Pharmacia)[14], followed by purification of the product by HPLC, as above. Its structure was verified by UV spectroscopy and conversion to G3'p5'G and ^{32}Pi with alkaline phosphatase.

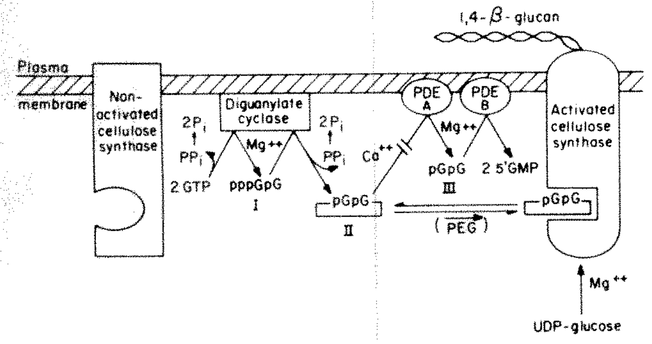

Fig. 4 Proposed model for regulation of cellulose synthesis in *A. xylinum*. Diguanylate cyclase, presumably a membrane-associated enzyme, converts two molecules of GTP into a 5′ triphosphate dimer (I) which is then condensed intramolecularly to form cyclic diguanylic acid (II). The pyrophosphate released in each step is rapidly hydrolysed to inorganic phosphate. Association of cyclic diguanylic acid with the cellulose synthase activates the enzyme. (*In vitro*, this interaction is promoted by PEG.) Deactivation occurs when a membrane-bound phosphodiesterase (PDE-A) cleaves the cyclic structure of the nucleotide to yield the inactive open dimer (III). A second phosphodiesterase (PDE-B) splits this product into two molecules of 5′ GMP. Calcium ions block the first of these degradative steps, thus preserving the nucleotide activator and extending its availability to the synthase. For simplicity, the synthesis of a single 1,4-β-glucan chain is depicted, although a more complex form of the synthase, polymerizing several chains simultaneously, might be the active enzyme unit in cellulose biogenesis.

synthase activity is markedly increased by the presence of either Ca^{2+} or polyethyleneglycol (PEG) (Fig. 1*a*). The effect of Ca^{2+} is attributed to its inhibition of the membrane-bound enzyme which degrades the activator (see below). The interaction between the activator and the synthase within the membrane is apparently reversible, since membranes exposed to c-di-GMP lose all their enhanced synthase activity upon washing, which can subsequently be restored upon readdition of the activator. Enhanced activity is not due to an increase in the affinity of the synthase for UDP-glucose, since the K_m for this substrate[2] (0.2 mM) is not affected by c-di-GMP. The digitonin-solubilized cellulose synthase[3] is similarly stimulated by c-di-GMP (Fig. 1*b*). In this case, however, stimulated activity is not affected by Ca^{2+} or PEG. The absence of the Ca^{2+} effect is compatible with our finding that the enzyme degrading the activator is destroyed by digitonin treatment. Although the mechanism of cellulose synthase activation by c-di-GMP is still unclear, it is meaningful that the 1,4-β-glucan product of the cyclic nucleotide activated synthase of *A. xylinum*, recently analysed by electron microscopy and electron diffraction[8], has been shown to consist of fibrillar cellulosic material (probably cellulose I).

The enzymatic degradation of CSA by membranous preparations of *A. xylinum* to 5′ GMP as sole final product, occurs by two distinct steps[5]. The initial degradation product arising from ^{32}P-labelled c-di-GMP has now been isolated and identified as the linear dimer pG3′p5′G on the basis of enzymatic[5] and sequential degradation by periodate/β-elimination[5,9] and by comparison to synthetically prepared (Fig. 3*b*) pG3′p5′G (data not shown). Thus, the first step of the pathway of CSA degradation involves hydrolytic opening of its cyclic structure. This step is strongly inhibited by Ca^{2+} (with an EDTA-free membrane preparation, this activity is inhibited 50% by 40 μM Ca^{2+}). On the other hand, the conversion of the linear dinucleotide to 5′ GMP is not affected by Ca^{2+}, which may indicate the involvement of two different enzymes in the degradation of the c-di-GMP. With the activator being a cyclic nucleotide, the action of its degrading enzyme(s) may then be similar in its site of hydrolysis and presumed physiological role to that of the specific phosphodiesterases acting on 3′,5′ cyclic AMP or 3′,5′ cyclic GMP.

A model summarizing the sequence of reactions involved in the regulatory mechanism of the cellulose synthase of *A. xylinum* is presented in Fig. 4. The pace of the polymerization process is set by the cellular levels of c-di-GMP which, in turn, depend on the concentration of cellular Ca^{2+}. The stability and unique structure of the nucleotide activator are compatible with its role as an intracellular messenger regulating cellulose synthesis *in vivo*. Although the regulation of cellulose synthesis has thus far been demonstrated only for *A. xylinum*, it is tempting to speculate that mechanisms similar to that described here, based on cyclic diguanylic acid or on related cyclic di- or oligonucleotides, may function in other organisms and other cellular processes. In this respect, it is interesting to note that the initiation reaction of RNA polymerase *in vitro*, under certain conditions, is accompanied by release of dinucleoside 3′,5′ tetraphosphates[10] and strongly inhibited by several synthetic cyclic 3′,5′ dinucleotides[11].

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Initiation of translation is impaired in *E. coli* cells deficient in 4.5S RNA

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The 4.5S RNA of *Escherichia coli* is a small, stable RNA that is essential for cell growth but its function is not yet known. Its biosynthesis is stringently controlled, and it is processed by RNase P, a transfer RNA processing enzyme[1–3]. To identify the biological role of the 4.5S species, we have characterized the physiological changes that occur when the bacterial cell is depleted of this RNA. We used a strain of *E. coli* in which synthesis of the 4.5S RNA can be turned off by removing an inducer of the *lac* operon, resulting in cell death[4]. We report here that an early consequence of depriving the cell of 4.5S RNA is the accumulation of translationally-defective ribosomes, which maintain their ability to elongate polypeptide chains, but can no longer participate in the initiation of protein synthesis.

Bacterial strains in which synthesis of 4.5S RNA is under the control of isopropyl-β-D-thiogalactoside (IPTG), an inducer of the *lac* operon, were constructed (see refs 4, 5 for details). A copy of the 4.5S RNA structural gene (*ffs*) was fused to an

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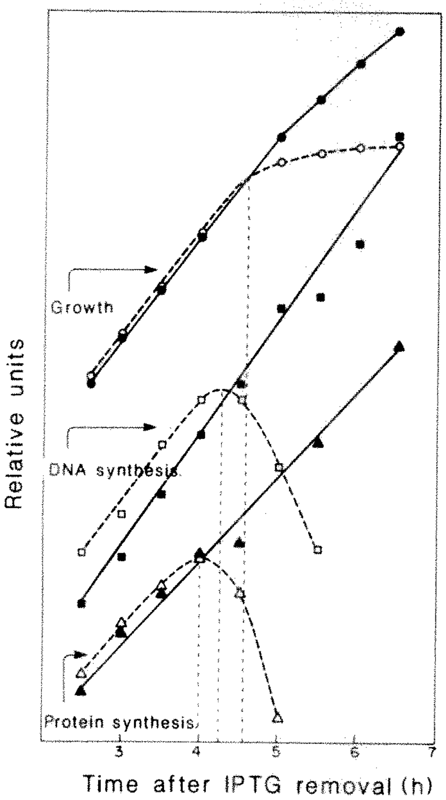

Fig. 1 Cessation of growth, DNA synthesis, and protein synthesis as 4.5S RNA is depleted. Strain FF242 was grown to saturation at 37 °C in minimal MOPS-glucose medium[16] supplemented with 10 mM IPTG. After being collected by centrifugation, the cells were washed twice with medium containing no IPTG. These cells were then used to inoculate 50 ml cultures with and without IPTG. Growth was monitored at 535 nm. At the times indicated during logarithmic growth, 300 μl of cells was removed from each culture and labelled for 5 min with either 0.1 μCi of [^{35}S]methionine (400 Ci mmol^{-1}), or 0.04 μCi of [^{14}C]thymidine (55.7 mCi mmol^{-1}). After labelling, 3 ml of cold 10% trichloroacetic acid (TCA) was added. Samples were heated at 90 °C for 15 min, and the acid-insoluble activity collected by filtration and measured by scintillation counting. Circles, turbidity with (●) and without (○) IPTG; squares, incorporation of [^{14}C]thymidine with (■) and without (□) IPTG; triangles, incorporation of [^{35}S]methionine (▲) with and (△) without IPTG.

IPTG-inducible *tac* promoter[6]. This *Ptac::ffs* fusion was inserted into the DNA of lambda phage *c*I857*nin*5 at the unique *Xho*I site. This phage was crossed with λ*imm434*, and the resultant λ*imm434 Ptac::ffs* phage was packaged and introduced into the genome of *E. coli* S1224 HfrH *lac*I$^{\mathrm{Q}}$ by lysogeny. The normal chromosomal *ffs* gene was then inactivated by partial replacement of the coding sequence with a kanamycin determinant[4], using phage P1 transduction to replace the 5′ two-thirds of the *ffs* gene by a gene encoding resistance to kanamycin. The resulting strain is designated S1192. Another strain, with the same *ffs* genotype, was constructed essentially as described, but with W3110 *lac*I$^{\mathrm{Q}}$ L8 (ref. 7) as the recipient of the recombinant phage. This second strain, called FF242, permits direct comparison with strain W3110 which has been widely used for growth and metabolic studies.

Withdrawal of IPTG results in cessation of growth as 4.5S RNA is depleted (ref. 4 and Fig. 1). Gel electrophoresis of RNA extracted from cells at various times shows that the amount of 4.5S RNA decreases several fold as IPTG deprivation continues (data not shown). Incorporation of labelled methionine into protein ceases 15–20 min before either DNA synthesis or cell growth is impaired. The relative immediacy of this effect, taken with the data on its regulation and processing, suggests that 4.5S RNA may be involved in protein synthesis.

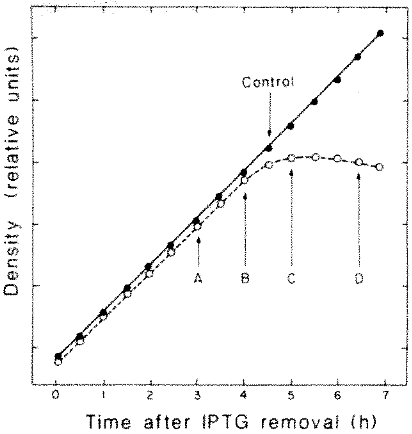

Fig. 2 Growth of cells with *lac*-controlled 4.5 S RNA synthesis. *E. coli* strain S1192 was grown to saturation at 37 °C in yeast extract and tryptone (YT) medium supplemented with 0.4% lactose, instead of IPTG. Cells were transferred to YT medium without lactose for 40 min at 37 °C, then diluted into YT medium with and without 10 mM IPTG, and growth was monitored. Density markings correspond to logarithmic units. ●, Turbidity with IPTG; ○, turbidity without IPTG; A, B, C, D, times of sampling.

Methods. At the times indicated, cultures were chilled, harvested, and quick-frozen. Frozen pellets were ground with twice their weight of alumina and resuspended in 1.5 ml of 10 mM Tris-acetate, *p*H 8.2, 14 mM magnesium acetate, 60 mM KCl, and 1 mM dithiothreitol (DTT) per g of cells. Extracts were centrifuged for 5 min at 5,000*g* to remove the alumina and cell debris, and supernatants centrifuged twice for 30 min at 30,000*g*. To the resulting supernatants (S-30) was added 160 μl per g of cells of 0.75 M Tris-acetate, *p*H 8.2, 0.021 M magnesium acetate, 7.5 mM DTT, 0.075 mM each amino acid, 6 mM ATP, 67.5 mM phosphoenolpyruvate, and 0.02 mg ml^{-1} pyruvate kinase. Extracts were incubated for 80 min at 37 °C in the dark, then dialysed overnight against two changes of 10 mM Tris-acetate, *p*H 8.2, 14 mM magnesium acetate, 60 mM potassium acetate, and 1 mM DTT. Extracts were quick-frozen at −80 °C in small aliquots.

The translational activities of extracts from 4.5S RNA-depleted cells were evaluated in the *in vitro* system of Zubay[8]. Extracts were prepared at various times following removal of inducer and compared with a control extract from cells maintained in the presence of IPTG. Figure 2 shows the growth rate of the cells with and without IPTG and the times at which the 4.5S RNA-depleted extracts were prepared. The translational activity of the various extracts was measured as described, using two types of RNA templates[8]. These were: poly(U), expression of which does not require the normal initiation process; and natural messenger RNA, consisting of endogenous mRNA present in the extracts and exogenous Q*β* phage RNA, for which the initiation events must occur. Figure 3*a* and *b* shows the results of testing various 4.5S RNA-depleted extracts for their ability to use each template. When poly(U) was present, all extracts were seen to have the same activity, suggesting that the capacity to elongate polypeptide chains is the same in control and 4.5S RNA-depleted extracts. However, when natural message was used, translational activity decreased as 4.5S RNA was depleted. As these extracts maintain the ability to elongate, it follows that initiation must be impaired in the depleted extracts.

We examined the structural integrity of the ribosomes in crude extracts using sucrose density gradient centrifugation. Ionic conditions were selected that do not promote association of free subunits or cause dissociation of 70S complexes[9]. Extracts dep-

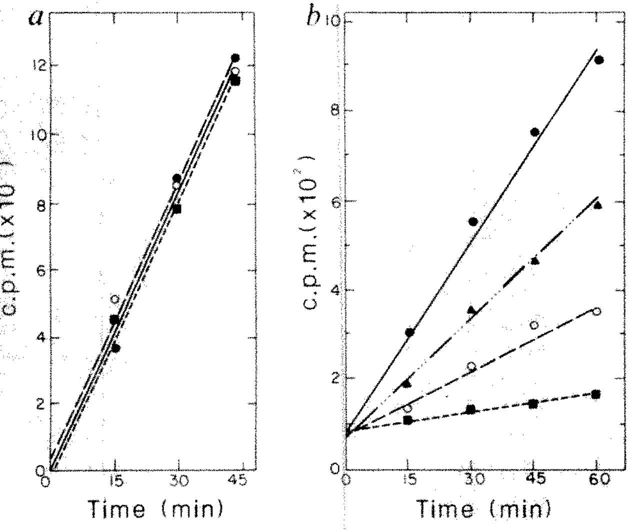

Fig. 3 *a*, Translational activity of control extract and 4.5S RNA-depleted extract using poly(U) as template, measured as incorporation of [^{14}C]phenylalanine. ●, Control extract; ○, extract C; ■, extract D. *b*, Translational activity of control extract and 4.5S RNA-depleted extract using natural message, measured as incorporation of ^{3}H-labelled amino acids (mix of all but asparagine, cysteine, glutamine, methionine and tryptophan). ●, Control extract; ▲, extract A; ○, extract C; ■, extract D.

Methods. Extracts were prepared as in Fig. 2, and 250 μg of the appropriate extract was added to a final volume of 200 μl, with 44 mM Tris-acetate *p*H 8.2, 55 mM potassium acetate, 27 mM ammonium acetate, 14.7 mM magnesium acetate, 7.4 mM calcium acetate, 1.37 mM DTT, 0.22 mM each asparagine, cysteine, glutamine, methionine and tryptophan, 2.2 mM ATP, 0.55 mM GTP, 21 mM Na$_3$PEP (phosphoenolpyruvate), 100 μg ml^{-1} *E. coli* tRNA, 27 μg ml^{-1} pyridoxine-HCl, 27 μg ml^{-1} NADP, 27 μg ml^{-1} flavin adenine dinucleotide (FAD), 11 μg ml^{-1} *p*-aminobenzoic acid, 27 μg ml^{-1} folic acid, 50 μg ml^{-1} of either poly(U) or Qβ RNA, and either 2 μCi [^{14}C]phenylalanine (513 mCi mmol^{-1}) or 0.2 μCi [^{3}H] labelled amino-acid mix (250 mCi ml^{-1} each amino acid). Extracts were added last, and 38 μl removed at various times into 1 ml of cold 5% TCA. Samples were heated at 90 °C for 15 min, and the acid-insoluble activity collected by filtration and measured by scintillation counting.

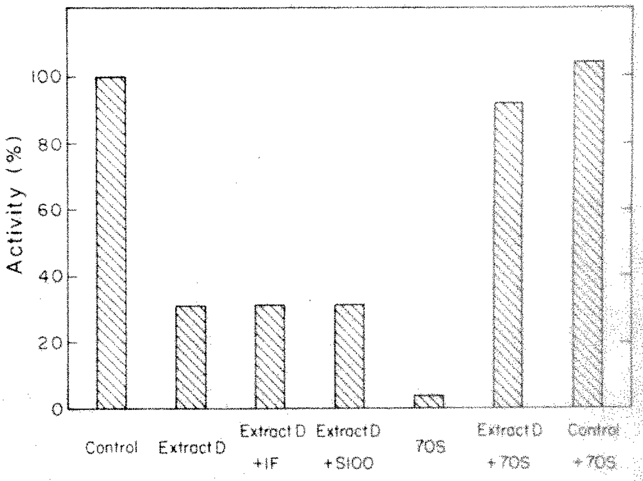

Fig. 4 Protein synthetic activity of a 4.5S RNA-depleted extract complemented with fractions of control extract.

Methods. Control extract was fractionated as follows: S-30 extract was spun at 40,000 r.p.m. for 4 h in a Beckman SW-60Ti rotor to pellet crude ribosomes. The supernatant was the S-150 fraction. The crude ribosomes were resuspended in 10 mM Tris-Cl, *p*H 7.6, 15 mM MgCl$_2$, 1.0 M NH$_4$Cl, 5 mM 2-mercaptoethanol, and incubated on ice for 1 h with occasional agitation. The washed ribosomes were collected by ultracentrifugation as above, and initiation factors (IF) were precipitated from the supernatant by the addition of 470 mg ml^{-1} (NH$_4$)$_2$SO$_4$ (ref. 17). This precipitate was collected by (ref. 17) centrifugation and resuspended in 10 mM Tris-Cl, *p*H 7.6, 20 mM NH$_4$Cl, 5 mM MgCl$_2$, 5 mM 2-mercaptoethanol, and dialysed overnight against the same. Aliquots of the IF fraction were then quick-frozen and stored at −80 °C. The washed ribosomes were resuspended in 10 mM Tris-Cl, *p*H 7.6, 15 mM MgCl$_2$, 60 mM NH$_4$Cl, 5 mM 2-mercaptoethanol, and pelleted again as above. Aliquots were resuspended in the same buffer, quick-frozen and stored at −80 °C. Activity of control extract was defined as 100%. Reactions were carried out as in Fig. 3, using Qβ mRNA, except that no time points were taken; after 30 min the entire reaction mix was added to cold 5% TCA. The following additions were made where indicated: 80 μg S-150, 80 μg IF, 80 μg washed 70S ribosomes.

leted of 4.5S RNA contained more free subunits than control extracts, consistent with the observation that initiation is impaired in depleted extracts. Although extracts from later time points showed a greater than fourfold increase in the level of free subunits, the magnitude of this effect does not correspond to the larger decrease (90%) observed in translational activity. This suggests that a much larger portion of the ribosome pool may be unavailable for initiation.

The reduction in translational activity can be explained by a defect within the components required for initiation, or by the presence of an inhibitor that accumulates as a consequence of 4.5S RNA depletion. To distinguish between these possibilities, control extract was fractionated further into a S-150 fraction, a 1 M salt ribosomal wash fraction containing initiation factors (IF), and salt-washed 70S ribosomes. These fractions were each added separately to the least active 4.5S RNA-depleted extract, and their ability to restore activity tested (Fig. 4). The S-150 and IF fractions, observed to be functional with control 70S ribosomes (data not shown), had no effect on the activity of the depleted extract. But, the addition of an amount of washed 70S ribosomes roughly equal to that present in control extract restored activity to the control level. As the same ribosomes had little effect on the control extract, we conclude that translation in the depleted extracts is limited by a lack of initiation-competent ribosomes. Apparently, the ribosomes become defective as 4.5S RNA is depleted.

The possibility that the functional defect in the ribosomes of 4.5S RNA-depleted cells could be correlated with a structural alteration was assessed by sucrose density gradient analysis. No difference in sedimentation was observed for either intact ribosomes or individual subunits from depleted extracts when compared with control ribosomal particles (data not shown). Thus, if a structural alteration is the basis of the effect, it does not result in grossly modified subunits, although more subtle alterations are not ruled out.

No increase in activity was observed when purified 4.5S RNA was added to depleted extracts, even in amounts 100-fold greater than that typically found in control or wild-type extracts. The 4.5S RNA normally accounts for 1–2% of the total small RNA in *E. coli*[10], and is present in extracts at about 1 μM. These results suggest that the 4.5S species does not act as a classic soluble factor in the initiation of protein synthesis. Nor does the 4.5S RNA seem to serve as an essential structural component of the ribosome, because the wild-type level of 4.5S RNA is approximately one tenth that of the 5S ribosomal RNA, and virtually all 5S RNA is ribosome associated[11]. Earlier experiments have shown that the 4.5S RNA is not associated with crude ribosomes[12,13].

Because the 4.5S RNA cannot be shown to restore activity to depleted extracts by itself, we cannot conclude that the observed effect is a direct result of 4.5S RNA depletion. However, less direct evidence argues strongly for the participation of this molecule in the protein synthetic process. 4.5S RNA is under stringent control, as are the ribosomal and transfer RNAs (tRNAs), and also like tRNAs, is processed by RNase P. It has a long lifetime, as do other RNAs involved in protein synthesis[13]. More direct is the observation that the earliest event following 4.5S RNA depletion is the appearance of initiation-incompetent ribosomes. Three hours after the removal of IPTG, which is roughly three generations, initiation activity is 65% that of control. The longevity of the molecule (longer than that of 5S RNA, ref. 13) suggests that this number of cell divisions must occur before dilution and turnover cause the cellular level to drop below that required for proper function. Also, there is no detectable alteration in the protein makeup of cells, as judged by two dimensional electrophoresis, until after initiation becomes impaired (D.B.B., T. A. Phillips, F. C. Neidhardt & M.J.F., in preparation). Patterns of labelled proteins remain constant until initiation activity drops below the 65% level. Decreases below this point are accompanied by an increase in expression of several proteins. There is no apparent accumulation of incomplete peptides. Taken together, these correlations support the view that impairment of translational initiation occurs as a direct consequence of the loss of 4.5S RNA.

The data suggest that the 4.5S RNA participates in creating or maintaining the viability of a ribosomal subunit. That ribosomal particles appear normal, as judged by the somewhat insensitive sedimentation assay, suggests that a late stage of assembly or maturation is affected. It is unlikely that ribosomal RNA maturation is affected by a lack of 4.5S RNA; if assembled, ribosomes containing unprocessed RNA would show altered sedimentation. Evidence that the 4.5S RNA is not a structural component comes from the fact that active ribosomal particles can be assembled *in vitro* from individual RNAs and proteins without 4.5S RNA being present[14,15]. Of course, this finding does not preclude the possibility that 4.5S RNA might have a catalytic role in assembly *in vivo*. Thus, 4.5S RNA may not be required for initial assembly or maturation, but for the development or maintenance of a functional form as subunits recycle into each initiation phase. Interestingly, the 4.5S RNA contains exposed sequences similar to the Shine-Dalgarno sequence of mRNA. Two such overlapping domains occur in the apex region of the predicted hairpin-like secondary structure and this region is particularly susceptible to nuclease (ref. 10 and B. Sneath, M.J.F. & J. N. Vournakis, in preparation). Perhaps the 4.5S RNA binds to the 3′ end of the 16S RNA and assists in mRNA binding or prevents the degradation of the 3′ end of the 16S RNA. In the absence of 4.5S RNA, the 3′ end of the 16S RNA could be degraded, resulting in an inability to participate in initiation.

Experiments are under way to determine which subunit contains the defect responsible for translational inactivation, which event in the initiation process is affected by this defect, and whether conditions can be found in which 4.5S RNA will restore activity to depleted extracts. Results from these experiments will allow the models discussed above to be tested.

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Erratum

A cell surface molecule distributed in a dorsoventral gradient in the perinatal rat retina

Martha Constantine-Paton, Andrew S. Blum, Rosalia Mendez-Otero & Colin J. Barnstable

Nature **324**, 459–462 (1986).

IN this letter the wrong Fig. 4 was used. This is the correct Fig. 4 and its legend, which is as published.

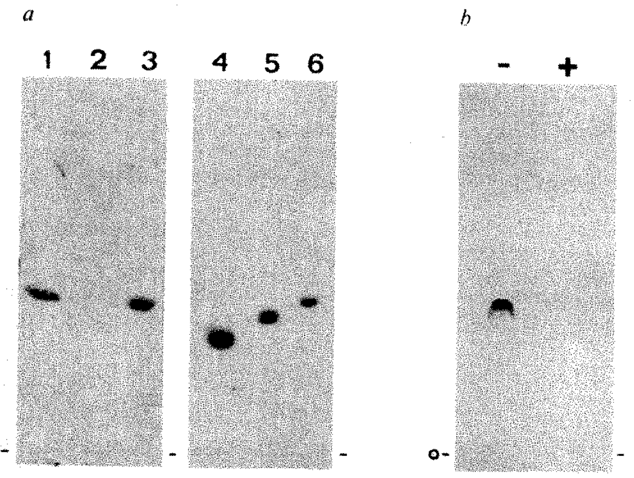

Fig. 4 Biochemical characterization of the JONES antigen using TLC/immunoblot analysis. *a*, The JONES antigen partitions into the aqueous phase of a Folch extract and is detected as a single band on immunoblots of lipids resolved by TLC. This band migrates to a position between that of G_{M2} and G_{M1} ganglioside standards run at the same time. (1) Whole lipid extract; (2) organic phase; (3) aqueous phase; (4) G_{D1a}; (5) G_{M1}; (6) G_{M2}. 1-3, Immunostaining of the nitrocellulose blot of a TLC plate; 4-6, resorcinol staining of purified standards run at the same time. *b*, The epitope recognized by JONES antibody contains sialic acid as it is removed by treatment with neuraminidase (+) but not buffer (−).

Methods. P4 rat retinas were homogenized in chloroform/methanol (2:1) to give a crude lipid extract[36]. This was partitioned into organic and aqueous phases by 0.1 M KCl[9]. Gangliosides and other highly polar lipids (including some phospholipids) partition into the aqueous phase, whereas most neutral glycolipids and some less polar gangliosides partition into the organic phase. The sialic acid content of the fractions was determined by the resorcinol method[37]. Samples containing 0.3–0.4 μg sialic acid were applied to a precoated HPTLC plate (Merck, silica gel 60, 0.2 mm thick) and developed with chloroform/methanol/0.25% aqueous $CaCl_2$ (60:40:10). Chromatograms were dried, wetted with isopropanol/water (2:1) and blotted onto nitrocellulose[38]. The nitrocellulose blot was blocked in 5% normal goat serum and incubated with JONES antibody for 4 h. After washing, the blot was incubated in peroxidase-conjugated goat anti-mouse IgG (H+L) for 2–4 h and washed. Bound antibody was visualized by incubation with 3.3′ diaminobenzidine. Ganglioside standards were run on parallel lanes as controls and were visualized by resorcinol staining[37]. For neuraminidase digestion the aqueous phase retinal lipids were dried under N_2 and resuspended in 0.1 M sodium acetate, *p*H 5.5, with or without neuraminidase (Sigma type X from *Clostridium perfringens*) at 8 mU per μg sialic acid. Incubations were carried out for 15 h at 37 °C. Digested lipids were extracted into chloroform:methanol, 2:1, dried under N_2 and resuspended in a small volume of chloroform:methanol, 2:1. Samples were run and analysed by TLC/immunoblot as described above.

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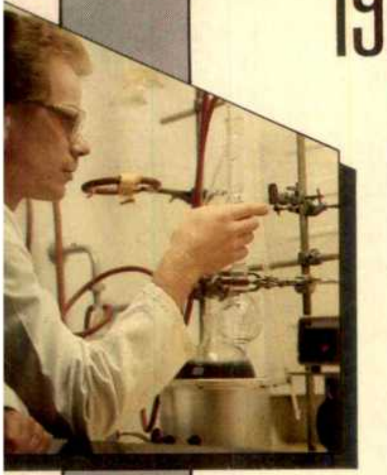

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Your Careers Centre should have information about Research at Glaxo, and completed application forms should either be returned to the Careers Centre or sent to Mr R. W. Jones, Graduate Recruitment Co-ordinator, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex, UB6 0HE. Forms can also be obtained from this address if necessary.

Please Note. We may not be scheduled to visit your University or Polytechnic but, naturally, we want to hear from you. Please write to the above address for details and an application form.

Glaxo Group Research Ltd.

(1785)A

UNIVERSITY OF QUEENSLAND
Brisbane, Australia

Applications are invited for the following positions:

Queensland Institute of Medical Research

Education and Research Positions in Tropical Health

The Medical Faculty, University of Queensland and the Queensland Institute of Medical Research, Brisbane will jointly start in 1987 a new programme of postgraduate research and training in Tropical Health. This programme is funded by the Commonwealth Department of Health, Canberra and is a substantial expansion of existing international commitments in this field. Academic positions, the majority of which will be joint appointments, are hereby advertised in the following professional areas.

Tropical Epidemiology and Public Health
Tropical Medicine, with emphasis on Primary Health Care
Medical Anthropology, Socio-Cultural Anthropology,
with interest in health care
systems in developing countries
Medical Parasitology
Medical Entomology
Microbiology
Public Health Administration in Developing Countries
Medical Statistics
Molecular Biology/Immunology of Tropical Diseases

Academic/Research ranks will be decided according to the achievements and experience of the successful applicants. One of the senior, professional appointments will also be that of Director of the Master's Degree and Diploma Course in Tropical Health. A condition of appointment will be that appointees be prepared to undertake a substantial period of work each year in the tropical countries that have partner institutions in this programme. This period will be spent in field research, and/or supervising the practical field work of the students, and/or sharing in the academic development of those institutions.

Applications close 16th February 1987. Further information, written or oral, on each vacancy can be obtained from the Dean, Faculty of Medicine, Medical School, Herston, Brisbane, Queensland 4006, Tel: (07) 253 5151 and the Director, Queensland Institute of Medical Research, Brisbane 4006 (07) 253 6202. Ref: 51986. Application forms also available from the Director.

Lecturer/Senior Lecturer in Mathematical Statistics

Department of Mathematics

Should have a higher degree together with teaching and research experience in one or more branches of mathematical statistics. Two tenurable positions will be available: one to be filled early in 1987 and the other after 6 July 1987.

Closing date 30th January 1987, or as soon as possible thereafter. Ref: 51186.

Salary per annum: Senior Lecturer $A37,381 - $A43,568, Lecturer: $A27,859 - $A36,600.

Additional information about this post and application forms available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University of Queensland is an Equal Opportunity Employer
(W3734)A

Centre National de la Recherche Scientifique
Laboratoire de Physiologie Nerveuse

Molecular Biologist/Biochemist

Postdoctoral position for up to 3 years to work on neuropeptides biosynthesis or on oncogenes in brain.

Previous experience with yeast will be appreciated. Salary negotiable according to experience. Applications containing a full curriculum vitae and the names and addresses of two referees should be addressed to: **Dr Pascal Madaule or to Dr Jean Rossier, Laboratoire de Physiologie Nerveuse, C.N.R.S. 91190 Gif-sur-Yvette, France.**
(W3717)A

RE-ADVERTISEMENT FOR UPGRADED POSTS

Agricultural and Food Research Council
Institute for Animal Disease Research
Houghton Laboratory

MOLECULAR BIOLOGISTS

We are seeking to fill the following vacancies:

Ref. NI 111/1: The Institute has a vigorous programme of research in molecular biology related to disease control in poultry. Applications are invited from experienced molecular biologists to join the genetics group which is working on the genes of the major histocompatability complex of the domestic fowl — candidates should have experience in cloning and determining expression of eukaryotic genes using cosmid and lambda vectors and it would be useful if they had experience on the identification of mRNA species transcribed from eukaryotic genes. The post is tenable for two and a half years.

Ref. NI 215/4: A molecular biologist to join a group within the parasitology department engaged in the development of a genetically-engineered vaccine against coccidiosis. The project will involve the development of novel live vaccine vectors and assessment of the immunity induced in chickens by a variety of constructs designed to optimise the expression and localisation of eimeria antigens. Some experience in molecular biology, microbial genetics and/or immunology would be an advantage. The post is tenable for a period of three years.

Applicants should have a first or upper second class honours degree in appropriate subjects and preferably a PhD with at least two years relevant postgraduate experience for Higher Scientific Officer grade, and four years for the Senior Scientific Officer.

Salaries: Higher Scientific Officer — £8,405×6 to £11,323
Senior Scientific Officer — £10,558×7 to £13,762

For further particulars and application forms please contact the Assistant Secretary at the Institute for Animal Disease Research, Houghton Laboratory, Houghton, Huntingdon, Cambs PE17 2DA — Telephone St Ives (0480) 64101 quoting the appropriate reference number.
Closing date for applications is 6th February 1987.

The Institute for Animal Disease Research is an Equal Opportunities Employer. (1786)A

IGNEOUS/METAMORPHIC PETROLOGIST:

This is a new faculty position, twelve-month appointment, tenure-track, currently 25% funded from State support. Candidate is expected to develop a strong research program, applying theoretical, experimental, and/or latest analytical methods to geochemical/petrological problems; particularly those related to the origin and evolution of oceanic crust, islands, and/or volcanic arcs. Research experience with one or more of the following is desirable: XRF, XRD, ICP/DCP, stable isotopes, solid source mass spectrometry (Sr, Pb, Nd). Rank to be Assistant Professor. Resume, names, addresses and phone numbers of three references must be received by 10 March 1987 by **Douglas R. Caldwell, Dean, College of Oceanography, Oregon State University, Corvallis, OR 97331.** *Oregon State University, an AA/EO employer.* (NW372)A

UNIVERSITY OF WASHINGTON, SEATTLE

POSTDOCTORAL POSITION

A position is available to study the regulation of expression of genes coding for extracellular matrix proteins. Two human genes, one coding for a subunit of type I collagen and the other for thrombospondin have been chosen for detailed study. Of particular interest are the mechanisms by which growth factors influence the expression of these genes. Applicants with some previous experience in molecular biology are preferred. Applicants should send a resume and the names of three references to: **Dr Paul Bornstein, Department of Biochemistry, SJ-70 University of Washington, Seattle, WA 98195.** *The University of Washington is committed to Equal Opportunity through Affirmative Action in employment.* (NW360)A

Procter & Gamble

OUTSTANDING CAREER OPPORTUNITIES IN EUROPEAN RESEARCH & DEVELOPMENT

THE COMPANY

Procter & Gamble is a leading international Company, with a worldwide reputation for developing, manufacturing and marketing high quality consumer goods. In Europe, the Company has built a strong business in soaps, detergents, paper, food, beverages, personal care and health care products.
Procter & Gamble is technology based. Its commitment to R&D goes back to 1887, when it established one of the first R&D labs in U.S. industry. Today it is a leader in R&D investment in the consumer products business.

A P&G CAREER

The Company is well known for its excellent management and the development of its people. When you start your career at P&G, you will get immediate responsibility for challenging and meaningful work. You will have "state-of-the-art" equipment, and you will work with professional staff and leading scientists. Your academic degree is a foundation for expanding both technical and managerial skills. Promotion is from within; all our senior managers started at entry level.
Within our European Technical Center, we have career opportunities for

GRADUATES and Ph.D's

in CHEMISTRY - LIFE SCIENCES - CHEMICAL ENGINEERING

The specific subject of your research is not critical because we have a range of opportunities in the following fields:

PRODUCT DEVELOPMENT

Develop new product formulations to meet important consumer needs. Work with technical and business managers to launch successfully new products into the market.

PROCESS DEVELOPMENT

Develop new process technologies, delivering superior product quality at competitive cost. Work within project teams to ensure successful start-up in the plants.

ENVIRONMENTAL SAFETY

Design and coordinate programs to examine chemical and biochemical fate of chemicals released into the environment. Be the Company's main resource on environmental issue management. A Ph.D. degree is required.

HUMAN SAFETY

Design and execute toxicological programs which enable decisions on product and ingredient safety to humans. Obtain internal and external scientific review by leading experts and industry associations. A Ph.D. degree is required.

PERFUME DEVELOPMENT

Design new product fragrances based on a clear understanding of consumer tastes and the application of up-to-date technologies. Work within a world-team of perfumers, with product development managers and with outside perfume houses.
Intensive training is foreseen to become an expert perfumer.

We are looking for EEC nationals who: ☐ have strong technical competence and leadership qualities ☐ communicate clearly and persuasively ☐ can work effectively with supervisors, peers and subordinates ☐ have the desire and the ability to initiate change for the better.

These opportunities are available at our European Technical Center near Brussels, which is the Management and Technical Headquarters for Procter & Gamble's European Division.

If you are interested in any of these opportunities, please send your C.V. to
E. Daenen, Recruiting Manager,
Temselaan 100, B-1820 Strombeek-Bever.

(W3723)A

PROCTER & GAMBLE EUROPEAN TECHNICAL CENTER

Pampers ☐ Luvs ☐ Ariel ☐ Mr. Proper ☐ Dash ☐ Dreft ☐ Lenor ☐ Vizir ☐ Petrole-Hahn ☐ Camay ☐ Gini ☐ Splendid Coffee ☐ Valensina ☐ Vicks

National University of Singapore

FACULTY OF MEDICINE
DEPARTMENT OF ANATOMY

APPLICATIONS ARE INVITED FOR TEACHING APPOINTMENTS IN THE DEPARTMENT OF ANATOMY

Candidates should, in addition to an approved basic medical or dental degree, possess a PhD Degree or a recognised higher professional qualification. Preference will be given to candidates with teaching experience in gross anatomy and one of the following specialities: Embryology, Genetics, Histology, Neuroanatomy. A good research record will be a decided advantage.

Gross annual emoluments range as follows:

Lecturer	S$28,300—58,680
Senior Lecturer	S$53,160—94,090

(ST£1=S$3.13 approximately)

The commencing salary will depend on the candidate's qualifications, experience and the level of appointment offered. In addition, appointees with an approved basic medical or dental degree and relevant higher academic/professional qualification may opt to retain consultation fees up to 60% of their annual gross salary or to receive a fixed annual clinical allowance as follows:

Lecturer	S$6,000/9,000
Senior Lecturer	S$15,000

Leave and medical benefits will be provided. Depending on the type of contract offered, other benefits may include: provident fund benefits or an end-of-contract gratuity, a settling-in allowance of S$1,000 (single) or S$2,000 (married), subsidised housing at nominal rentals ranging from S$100 to S$216 p.m., education allowance for up to three children, subject to a maximum of S$10,000 per annum per child, passage assistance and baggage allowance for the transportation of personal effects to Singapore.

Application forms and further information on terms and conditions of service may be obtained from:

The Director
Personnel Department
National University
of Singapore
10 Kent Ridge Crescent
Singapore 0511

NUS Overseas Office
Singapore High Commission
in London
5 Chesham Street
London SW1
U.K.
Tel: (01) 235-4562

(W3718)A

NERC INSTITUTE OF VIROLOGY

Project Leader

Oxford

Applications are invited for a project leader to develop an independent research programme in the Institute of Virology, Oxford. Present research interests at the Institute include the genetic engineering of virus insecticides, studies of plant, insect and animal viruses, tick transmitted viruses, virus pathogenicity, comparative virology, the field application of virus insecticides, virus expression vectors and the coding strategies of RNA viruses.

Applicants should have a higher degree in an appropriate scientific discipline (Ph.D., DVM, D. Phil., MD) and are expected to have at least 2-3 years postdoctoral experience. Candidates should provide a curriculum vitae and brief statement of present and future research interests and plans. Salary will be in the Senior Scientific Officer range of £10,558 - £13,762 according to age and qualifications.

Application forms may be obtained from, and should be returned to, the Director, Professor D H L Bishop, Institute of Virology, Mansfield Road, Oxford OX1 3SR. Telephone: Oxford 512361. Closing date is 31st January 1987.

The Natural Environment Research Council is an equal opportunities employer. All jobs in the UK are open to men and women of all ethnic groups and the council also welcomes applications from disabled people.

(1802)A

Natural
Environment
Research
Council

Lothian Health Board

TEMPORARY BASIC GRADE CYTOGENETICIST

Royal Hospital for Sick Children, Edinburgh, from 1st February - 25th October, 1987, to cover maternity leave. Telephone Miss P M Ellis 031 667 1991 ext 272 for further details.

Typewritten curriculum vitae and the names and addresses of 2 referees to the Personnel Officer, Lothian Health Board, 11 Drumsheugh Gardens, Edinburgh, EH3 7QQ as soon as possible. Quote M/7.

(1787)A

UNIVERSITY OF LONDON

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST. THOMAS'S HOSPITALS

GUY'S CAMPUS

IMPERIAL CANCER RESEARCH FUND POSTDOCTORAL FELLOW IN BIOCHEMISTRY

Applications are invited for this new appointment in the Imperial Cancer Research Fund Clinical Oncology Unit to work with the Department of Radiological Sciences at Guy's Hospital. The oncology programme at Guy's is broadly based and there is a major research programme in breast cancer which includes epidemiology, endocrinology, histopathology, studies of diagnostic techniques and treatment trials.

The postdoctoral fellow will work on the application of Magnetic Resonance Spectroscopy to the study of human breast cancer. Other members of the team include a senior biochemist, a physicist and technical staff, and there will be close involvement of clinical colleagues. Preliminary studies will be aimed at defining the spectral features in cell lines and xenografts. Further studies will be aimed at identifying the changes in spectral characteristics attributable to treatment with the intention of establishing criteria indicative of sensitivity to treatment. Adequate resources exist within the Imperial Cancer Research Fund for these studies to be complemented by appropriate *in vitro* studies.

The work will be carried out on both a Bruker AM400 spectrometer and a Philips 1.5T whole body Gyroscan. The post is funded for three years in the first instance, and will have a salary scale of £8,020—£9,880 plus £1,297 London Weighting, depending on age and experience. For further information, contact Dr M.A. Richards or Dr P. Garlick on 01-407 7600, Exts. 3784 & 2433 respectively. Applications with c.v. to Dr Richards, Clinical Oncology Unit, Guy's Hospital, London, SE1 9RT by 20th February 1987. (1782)A

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF EARTH SCIENCES

GEOPHYSICAL FLUID DYNAMICS, TRACE ELEMENT GEOCHEMISTRY AND OTHER RESEARCH AREAS

Applications are invited from suitably qualified men and women for appointment to the following positions: Postdoctoral Fellow, Research Fellow, Senior Research Fellow in Geophysical Fluid Dynamics and Trace Element Geochemistry; also Postdoctoral Fellow in Environmental Geochemistry, Geochronology and Isotope Geochemistry, Geodymanics, Ore-Genesis Studies, Petrochemistry, Petrophysics or Seismology. Detailed information available on enquiry. PhD required at PDF level, at least years postdoctoral at RF level, 8 years postdoctoral at SRF level.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range:

Postdoctoral Fellow Grade 1 (at a fixed point) $A24,013—$A27,507 p.a.; Research Fellow $A27,859—$A36,600 p.a.; Senior Research Fellow $A38,917—$A46,420 p.a.; Appointment will be Senior Research Fellow/Research Fellow normally up to three years with the possibility of extension to maximum of five years; Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years. Grants are provided towards travel and removal. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should send resume, names and addresses of three to five referees and statement of research interests to the Registrar. Quote reference ES. 11.12.1.

Applications should be submitted to the undersigned by 6 March 1987 but late applications may be considered. Applications should be submitted to the Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia, from whom further particulars are available. Further particulars also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an equal opportunity employer.

(W3696)A

OSU

ENDOWED CHAIR IN HYDROGEOLOGY

The Ohio State University

The Department of Geology and Mineralogy seeks applications and nominations for the new **Ohio Eminent Scholar Chair in Hydrology.** This position is supported by an endowment of $1.25 million provided by the Ohio Board of Regents and The Ohio State University. Start-up funds are included in the appointment for research support for several graduate students and for a new fully-equipped water-chemistry laboratory staffed by a full-time technician. The position will be at the rank of Professor.

The Eminent Scholar must have a distinguished record of scholarship with international eminence in several areas of hydrogeology, such as:

- Development of numerical models for flow and solute transport.
- Development of stochastic flow models for fractured media.
- Development of geochemical equilibrium models describing hydrochemical facies relationships.
- Application of hydrogeologic and geochemical models to aquifer restoration and contaminant transport problems.
- Determination of the origin, migration, and possible economic utilization of subsurface brines.
- Development of models for transport and deposition of metallic and non-metallic mineral systems.

The appointee will be expected to contribute to the research and instructional program of the Department, to participate actively in the further development of its Hydrogeology Program, and to establish close working relationships with other faculty in the Department and on campus, as well as with federal, state, and municipal agencies, and private industry.

The Eminent Scholar will join a department of 23 full-time, 13 emeritus, and 9 adjunct faculty whose instructional and research activities include hydrogeology, geochemistry, geophysics, marine hydrology, paleobiology, mineralogy, glaciology, Quaternary geology, tectonics, biostratigraphy, petrology, economic geology, and sedimentology. The Ohio State University maintains interdisciplinary research institutes and centers whose work is concerned with various aspects of ground-water and surface-water hydrology.

The position is available immediately. The search will continue until an appointment is made. Interested persons are urged to request the prospectus on the position. Enquiries should be directed to: **Dr. Peter N. Webb, Chairman, or Dr. E. Scott Blair, Department of Geology and Mineralogy, The Ohio State University 125 S. Oval Mall, Columbus, Ohio, 43210-1398. Telephone (614) 292-2712.**

The University is an Equal Opportunity/Affirmative Action Employer

(NW179)A

MOLECULAR NEUROBIOLOGY

Institute for Advanced Biomedical Research

The Oregon Health Services University

Applications are invited for a senior level appointment in the general area of molecular neurobiology. This position will be on the tenure track and will carry affiliate appointments as associate or full professor in one of the basic science departments of the medical school. An equivalent appointment will be made in the Institute.

The research interests of the Institute focus on the cellular and molecular bases of nervous system function. Of particular interest are applications from researchers applying modern molecular genetic techniques to analyze the development or function of the nervous system in either vertebrates, invertebrates or simple model systems.

Generous start-up packages are available and laboratory space will be assigned in the Institute's recently completed 80,000 square foot facility on the OHSU campus.

Although there are no formal teaching obligations, members are encouraged, through affiliate appointments, to participate in the graduate education program of the School of Medicine. Included will be the opportunity to participate in the further development of the Institute, in the development of interdisciplinary research programs in molecular and cellular neurobiology, and in setting up graduate and postdoctoral training programs jointly with the basic science and clinical departments of the medical school.

Applicants should have an established, independent research program, a record of extramural research funding and the promise of further scientific accomplishment. Applications should include a curriculum vitae with a description of current research support, the names of at least three references, selected reprints, and a description of current and future research goals. Applications should be submitted by April 1, 1987 and addressed to: **Dr Michael Forte, Molecular Neurobiology Search Committee, Institute for Advanced Biomedical Research, The Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Road, Portland, Oregon 97201.**

The OHSU is an equal opportunity/affirmative action employer. (NW368)A

THE PETER MacCALLUM CANCER INSTITUTE
PETER MacCALLUM RESEARCH LABORATORIES
MELBOURNE, AUSTRALIA

MOLECULAR SCIENCE GROUP

POSTDOCTORAL SCIENTISTS

(Five positions)

The Molecular Science Group is actively engaged in research on the molecular biology of radiation damage and cellular transformation mechanisms, and the molecular pharmacology of drug/DNA interactions. Vacancies exist for five postdoctoral scientists to work on the following grant-funded projects. Applications from graduates with particularly relevant experience will be considered.

1 Investigation of the interaction between homologous sequences as an approach to specific gene inactivation. Experience in cell culture techniques (preferably including transfection) and/or general molecular biology is required. Funded by the National Health and Medical Research Council. Initially for one year with possible extension to three years. Further details can be obtained from Dr. R. Martin.

2. The cloning and expression in bacteria of the SGA antigenic determinant. Experience in cDNA cloning is essential. Funded by Medical Innovations Pty Ltd. Initially for one year with possible extension to three years. Further information from Dr. T. de Kretser.

3 The molecular biology of oncogene-related proteins. Experience in cDNA cloning is essential. Supported by the Anti-Cancer Council of Victoria. Appointment for one year with possible extension to two years. Further information from Dr. T. de Kretser.

4. Study of the effects of DNA binding anti-tumour agents on eukaryotic gene expression. Experience in general molecular biological techniques including mRNA analysis is essential. Supported by the National Health and Medical Research Council for three years. Further information from Dr. T. de Kretser or Dr. L. Wakelin.

5. Study of interaction of novel alkylating agents with DNA. Experience in Maxam-Gilbert DNA sequencing techniques and/or kinetic and equilibrium measurements of DNA-ligand interactions will be an advantage. Funded by Australasian Drug Developments Pty. Ltd. for two years. Further information from Dr. L. Wakelin.

Positions available from February 15th, 1987. Salary range $A24,012-$A29,656. Applications, including full curriculum vitae, names of three referees and contact phone numbers, should be sent to the Personnel Officer at the address given below. Clearly specify project(s) applying for. Interstate and overseas applicants should include a contact FAX number, if available.

THE PETER MacCALLUM CANCER INSTITUTE

481 Little Lonsdale Street,
Melbourne, Vic. 3000,
Australia.
Phone: 61 3 602-1333
Fax Aust. 61 3 67-3357

(W3724)A

PROTEIN ENGINEERING at GENENCOR

POSTDOCTORAL POSITION — Xray Crystallography

Applications are invited for a two year post-doctoral position to work with the protein engineering team at Genentech and Genencor. We seek a selfmotivated individual having experience in Xray crystallography. The successful applicant will have an opportunity to determine the three dimensional structure of a novel protein and continue the analysis of the structural consequences of site-specific mutagenesis in subtilisin. He or she will join an established group of protein crystallographers at Genentech having a sealed tube generator with precession cameras, an Enraf-Nonius CAD 4 diffractometer, VAX 11/785 computer, Evans and Sutherland PS300 and access to a Xentronics multiwire area detector. The position is funded by Genencor, a biotechnology joint venture of Genentech, Corning Glass, and the A.E. Staley Co. Both Genencor and Genentech strongly encourage publications and provide a stimulating environment for research. This position will be available January 1, 1987. Applications, including a curriculum vitae, list of relevant publications, and the names of three referees should be sent to:

Dr. Richard Bott
Biocatalysis Department
Genentech, Inc.
460 Pt. San Bruno Blvd.
South San Francisco, CA 94080
Phone: (415)266-1331

(NW386)A

Important laboratoire pharmaceutique en Région Parisienne

recrute

Chercheur pharmacologue biochimiste HF

intéressé à la pharmacologie de l'hypertension (système rénine-angiotensine, système nerveux sympathique).
De formation minimum 3ème cycle ou Ingénieur, expérimenté dans les domaines indiqués.

Adresser lettre manuscrite, CV + photo et prétentions, en précisant sur l'enveloppe la réf. 2952, à Média-System, 2 rue de la Tour-des-Dames 75009 Paris, qui transmettra.

média-system

(W3720)A

ASSOCIATE DIRECTOR

Banbury Center of Cold Spring Harbor Laboratory

In cooperation with the Preventive Medicine Institute/Strong Clinic, the Banbury Center is expanding its program of scientific meetings. The Associate Director will be responsible for organizing meetings on the origins and detection of human cancer. Information from these meetings will be disseminated via books for specialists and other publications for nonspecialist scientists and clinicians. The Associate Director will also be closely involved in the Center's other activities, which include meetings and publications in biotechnology and risk assessment.

The successful candidate will have excellent organizational skills and a good knowledge of one or more of the following: cancer research, biostatistics and biotechnology. Writing and publishing skills would also be desirable. The importance of the post will be reflected in the salary and conditions offered.

The Banbury Center is situated on the north shore of Long Island, forty miles from New York City.

Please send applications and curriculum vitae to:
Steve Prentis, Director
Banbury Center
Cold Spring Harbor Laboratory
P.O. Box 534
Cold Spring Harbor, N.Y. 11724

CSH Cold Spring Harbor Laboratory

An equal opportunity employer

(NW355)A

UNIVERSITY OF THE WEST INDIES

Mona, Jamaica

DEPARTMENT OF PHYSICS

LECTURER/ASSISTANT LECTURER

The appointment is to be made in the field of Electronics and the successful applicant should be able to teach and organise undergraduate courses in this area. It is further expected that the person appointed will be able to teach some general physics and will engage in the research activities of the Department. Duties to be assumed as soon as possible.

Salary scales: Lecturer J$33,660 ×1,110 — 40,320 (B)×1,188 — 47,448; Assistant Lecturer J$27,600×1,032 — 29,664. Up to five full economy class passages plus baggage allowance up to US$900 on appointment and normal termination. Special allowance up to US$300 for shipment of academic books and teaching/research equipment. Unfurnished accommodation at rental of J$360 per annum or housing allowance of 20% of pensionable salary plus 10% Mona housing allowance. UWI contribution of equivalent of 10% of pensionable salary to Superannuation Scheme. Subsidised Health Service. Annual Study and Travel Grant. Book Grant. Entertainment and Transportation Allowances totalling J$9,900 p.a. at Assistant Lecturer level and J$12,300 p.a. at Lecturer level.

Detailed applications (three copies) giving full particulars of qualifications and experience, date of birth, marital status, and names and addresses of three referees should be sent as soon as possible to the Campus Registrar, UWI, Mona, Kingston 7, Jamaica, W.I. Further particulars of the posts are available from the same source or from the Secretary General, Association of Commonwealth Universities (Appointments), 36 Gordon Square, London WC1H 0PF, to whom candidates in the UK should also send one copy of their applications.

(W3730)A

IMPERIAL COLLEGE

(University of London)

Department of Biochemistry

POSTDOCTORAL RESEARCH ASSISTANT

Required to work on a project supported by the National Kidney Research Fund to isolate and characterise a novel nephrotoxic fungal metabolite. Appointment for 18 months from 1 April 1987. Starting salary up to £9,802 pa (under review). Further particulars from, and applications with CV and names of 2 academic referees to, Dr P G Mantle, Department of Biochemistry, Imperial College, London SW7 2AZ.

(1794)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following position:

THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

EXPERIMENTAL NEUROLOGY UNIT

POSTDOCTORAL FELLOW

NEUROPHYSIOLOGIST/BIOPHYSICIST

An opportunity exists for a neurophysiologist/biophysicist to work on spinal cord/cerebral cortex function in mammals. The appointee will work in collaboration with Professor R. Porter on the neurophysiological basis of control of movement performance in primates and may also develop research in other aspects of motor function in mammals. The studies in the Experimental Neurology Unit are concerned with mammalian neurophysiology and biophysics of mammalian nerve cells and their synaptic interactions. These studies are relevant to the understanding of the functions of the brain and to the development of strategies for rehabilitation after nervous system damage or disease in man. Excellent facilities for neurophysiological investigations and for computer analysis of neurophysiological data exist. Closing date: 20 February 1987. Ref. JC 18.12.1.

Conditions of appointment: Salary will be in accordance with qualifications and experience within the range:

Postdoctoral Fellow Grade 1 (at a fixed point) $A24,013—$A27,507 p.a.; appointment will be normally two years, with the possibility of extension to maximum of three years.

Grants are provided towards travel and removal. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote the advertisement reference when requesting further particulars and when submitting applications, which should be forwarded to the Registrar, Australian National University, GPO Pox 4, Canberra, ACT 2601, Australia. Further information also available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. The University is an Equal Opportunity Employer.

(W3731)A

UNIVERSITY OF ILLINOIS
COLLEGE OF MEDICINE AT CHICAGO

DIRECTOR OF THE CENTER FOR CLINICAL MAGNETIC RESONANCE

The University of Illinois College of Medicine at Chicago is seeking a Director of its new Center for Clinical Magnetic Resonance. Given its longstanding biologic expertise in *in vitro* NMR spectroscopy, the University has an established, well equipped NMR laboratory in its Research Resources Center. The new Clinical Center for MR will focus on imaging and *in vivo* spectroscopy with a 1.5 tesla instrument and a 2.0 tesla instrument with a 30 cm bore for experimental *in vivo* spectroscopy. These two units are housed in a separate building physically connected to the University of Illinois Hospital, a 550 bed tertiary care facility serving Chicago, Illinois and the midwest region. The Center has 4500 gross square feet of dedicated research space and additional office and conference space.

The Director will be expected to lead an interdisciplinary research team and to develop the Center into a regional resource for clinical diagnosis of disease and for clinical and biomedical research training of health care professionals in magnetic resonance imaging and spectroscopy.

A candidate is expected to qualify for a senior appointment in a major clinical department in the College of Medicine, preferably the Department of Radiology. Significant experience in magnetic resonance imaging and spectroscopy and its medical applications and the M.D. degree or equivalent are prerequisites. Salary will be commensurate with experience and qualifications.

Nominations and applications should be submitted to: **Bruce H. Brundage, M.D., Chair, Search Committee, Professor of Medicine, University of Illinois, College of Medicine at Chicago, P.O. Box 6998, Chicago, IL 60680.**

The University of Illinois at Chicago is an affirmative action/equal opportunity employer. (NW371)A

MOLECULAR BIOLOGIST/ BIOCHEMIST/VIROLOGIST

We are looking for a *postdoctoral scientist* to work on an EEC funded project concerned with the construction of linear plasmids and artificial minichromosomes for genetic manipulation of mammalian cells. The project will use components of animal viruses and chromosomes to investigate the features necessary to maintain stability of extrachromosomal linear constructions after transfection into mammalian cells. This is a collaborative project with groups in the Universities of Tubingen and Rome and opportunities will arise for working visits between these laboratories.

The appointment will be made at the Higher Scientific Officer level. It will start on 1st April 1987 and continue for 2 years and 9 months. It is available within the Division of Molecular Biology which comprises an active group of about 40 scientists and technicians working on various aspects of the structure and function of viruses.

Applicants should preferably have some background and experience in molecular biology, but enquiries from those with relevant qualifications in related disciplines would be welcomed.

HIGHER SCIENTIFIC OFFICER (HSO)

Minimum qualification:	PhD
Salary:	£8,405—£11,323
Annual Leave:	22 days

The above post participates in a non-contributory pension scheme and subsidised Institute housing may be available.

Informal enquiries can be made by phone (0483 232441) or letter to Dr C. Bostock. To apply please send curriculum vitae and names of two referees to:

Dr B.W.J. Mahy
Head, Pirbright Laboratory
Institute of Animal Disease Research
Ash Road
Pirbright, Woking
Surrey GU24 0NF
UK

(1798)A

Molecular Biologist

Cheshire

An experienced graduate is required to join a team of molecular biologists currently engaged on an exciting programme involving the isolation of novel genes encoding pharmacologically active proteins. The team is part of a larger multidisciplinary group including protein chemists, immunologists, oligonucleotide chemists and fermentation technologists.

In addition to a BSc in biochemistry, microbiology or protein chemistry, the ideal candidate will have at least two years experience with a strong research group and be expert in the isolation, manipulation and cloning of DNA fragments in E.coli. Experience with mammalian RNA isolation and nucleotide sequencing will be particularly advantageous.

The laboratories are located in attractive Cheshire countryside. Salaries and related benefits are competitive and reflect the importance the Division places on the recruitment of high quality staff. Generous assistance with relocation is available if appropriate.

Please write enclosing a detailed c.v. and quoting reference MB. to:-

The Personnel Officer,
ICI PLC, Pharmaceuticals Division,
Mereside, Alderley Park,
Macclesfield, Cheshire SK10 4TG.

ICI is an equal opportunities employer. (1818)A

Systematist and Herbarium Director

The Department of Botany, University of Minnesota, seeks an assistant or associate professor in experimental vascular plant systematics, preferably with interests in molecular and/or biochemical approaches. The successful candidate will be expected to maintain a vigorous research program in a modern area of plant systematics.

Teaching responsibilities may include undergraduate and/or graduate courses within the department. Up to a quarter of the appointee's time will be devoted to directing the University of Minnesota Herbarium. The responsibilities involve supervision of herbarium staff, including a collections manager, stimulation of the use of the collections within and outside the University, public relations, and fund raising.

Additional attributes sought are an interest in herbarium computerization, teaching knowledge of temperate flora, familiarity with operation of a large herbarium, and proven ability to attract research funds. A PhD and minimum of 3 years of post-PhD experience are required but 5 or more years of post-PhD experience are preferred.

The deadline for receipt of application materials is February 27, 1987. Please submit curriculum vitae, reprints of research articles, a statement of professional goals and research interests and objectives, and arrange to have 3 letters of recommendation sent to: **Dr David J. McLauglin, Department of Botany, 220 Biological Sciences Center, 1445 Gortner Avenue, St. Paul, MN 55108.**

The University of Minnesota is an Equal Opportunity Educator and Employer and specifically invites and encourages applications from women and minorities. (NW362)A

MOLECULAR EPIDEMIOLOGIST

We are looking for a person who is interested in applying molecular techniques to the analysis of the epidemiology of African swine fever. This disease is caused by a large double-stranded DNA virus similar to, but distinct from, the poxviruses. Different isolates can be distinguished and identified on the basis of the fragments produced by digestion with restriction endonucleases. The project will be use these molecular makers to define the viruses in domestic and wild animal populations in different regions of Zambia, to follow individual virus types in field isolates collected from outbreaks of the disease and to follow changes in particular virus isolates during experimental infections under laboratory conditions. The project is supported for 3 years by funds from the Overseas Development Administration.

The appointment will be made as soon as possible at either the Veterinary Research Officer or Senior Scientific Officer level. The post would be suitable for qualified veterinarians or postdoctoral scientists, who have an interest in the application of new techniques to the study of exotic animal disease.

VETERINARY RESEARCH OFFICER (VRO)

Minimum qualifications:	Veterinary degree + 2 years postgraduate experience, MRCVS or equivalent.
Salary:	£13,498—£17,950
Annual Leave:	25 days

SENIOR SCIENTIFIC OFFICER (SSO)

Minimum qualifications:	PhD + 4 years postdoc. experience
Salary:	£10,558 — £13,762
Annual Leave:	25 days

The above post participates in a non-contributory pension scheme and subsidised Institute housing may be available.

Informal enquiries can be made by phone (0483 232441) or letter to Dr C. Bostock or Dr P. Wilkinson. To apply send curriculum vitae and names of two referees to:

Dr B.W.J. Mahy
Head, Pirbright Laboratory
Institute of Animal Disease Research
Ash Road
Pirbright, Woking
Surrey GU24 0NF
UK

(1797)A

RESEARCH OPPORTUNITIES

The **Mountain Research Station, Institute of Arctic and Alpine Research, University of Colorado,** offers support for graduate thesis research in the Front Range of the Rocky Mountains. We also invite applications from scientists who wish to conduct externally-funded research. Collaboration, teaching, and Research Associate status are possible for those with postdoctoral experience. The Station is at 2900 m near Boulder, Colorado, and provides access to environments from 1500 to 4300 meters. The University of Colorado at Boulder is an Affirmative Action/Equal Opportunity Employer. Enquiries to: **Dr Mark Noble, Mountain Research Station, Univerity of Colorado, Nederland, CO 80466, USA.** (NW370)A

Igneous/Metamorphic Petrologist

This is a new faculty position, twelve-month appointment, tenure-track, currently 30 per cent funded from State support. Candidate is expected to have established a strong research program, applying theoretical, experimental, and/or latest analytical methods to geochemical/petrological problems; particularly those related to the origin and evolution of oceanic crust, islands, and/or volcanic arcs. Research experience with one or more of the following is desirable: XRF, XRD, ICP/DCP, stable isotopes, solid source mass spectrometry (Sr, Pb, Nd). Rank to be Associate Professor. Resume, names, addresses and telephone numbers of three references must be received by 10 March 1987 by: Dr Douglas R. Caldwell, College of Oceanography, Oregon State University, Corvallis, OR 97331.

Oregon State University is an AA/EO employer.

(NW373)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(UNIVERSITY OF LONDON)

DEPARTMENT OF MEDICAL MICROBIOLOGY

MOLECULAR BIOLOGY POSTDOCTORAL RESEARCH ASSISTANT

A three year position, funded by the Cancer Research Campaign, is available from 1 April 1987 to investigate the association between hepatitis B virus infection and primary liver cancer. The successful applicant will join a research team associated with the Wolfson Unit of Molecular Medical Microbiology and Parasitology, a group of young and enthusiastic molecular biologists with interests in viruses and unicellular parasites. Experience in the construction of genomic and cDNA libraries and/or DNA sequencing would be advantageous.

The salary is on the Research Fellow 1A scale, currently £8,020 to £12,780 per annum plus £1,297 London Allowance (with USS membership).

Further details may be obtained from Dr Tim J. Harrison on 01-636 8636 ext 411. Applications should be sent to The Personnel Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, quoting ref. TH1. Closing date: 6 February 1987. (1805)A

UNIVERSITY OF MANCHESTER

CHAIR OF PATHOLOGY

The University invites applications from suitably qualified candidates for appointment to a Chair of Pathology. Candidates should possess a qualification registrable with the General Medical Council, have a proven record of excellence in research in any area of pathology and substantial experience in teaching, administration and the provision of histopathology services. The Central Manchester Health Authority is prepared to offer an honorary consultant contract to the successful, suitably qualified, applicant. The salary will be in the normal professorial range for full-time clinical appointments with superannuation.

Applications (one copy suitable for photographic reproduction) giving full details of qualifications and experience together with the names and addresses of three persons to whom reference may be made, should be sent to the Registrar, The University, Manchester M13 9PL, from whom further particulars may be obtained, and be received by him not later than February 27th, 1987. Please quote reference 281/86/NA. Overseas candidates may apply by air mail letter in the first instance. (1773)A

THE UNIVERSITY OF MELBOURNE

RESEARCH FELLOW GRADE 1 (TEMPORARY)

in the

SCHOOL OF PHYSICS

A Research Fellow Grade 1 is required to work on the ARGC funded project "The Potential Approximation in Nuclear Physics", the Chief Investigator of which is Professor B.H.J. McKellar. The appointee will work on the application of field theory and quark models in nuclear physics. A PhD in theoretical particle or nuclear physics is required.

The appointment will be for fourteen months from the date of appointment in 1987 in the first instance, but may be extended if the grant is renewed.

Further details may be obtained from Professor B.H.J. McKellar (03) 344-5122.

Salary in the range A$24,013—A$27,507 per annum.

Further printed information regarding details of application procedures and conditions of appointment is available from the Appointments Officer (Academic), University of Melbourne, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications, in duplicate, including names and addresses of at least three referees, and quoting position number 640 A434 should be addressed to the Director, Personnal Services, The University of Melbourne, Parkville, Victoria, 3052 Australia and close on 28 February 1987.

Equal opportunity in employment is the policy of The University of Melbourne. (W3732)A

UNIVERSITY OF MANCHESTER

Department of Rheumatology

POSTDOCTORAL RESEARCH ASSOCIATE IN BIOCHEMISTRY

Applications are invited for this post, funded by the Peter Kershaw Trust and tenable for up to three years, from candidates with a special interest in the biochemistry of the fibrinolytic system and micro-vascular disorders. The studies will be directed towards chronic inflammation in rheumatic diseases and in particular spinal problems.

Salary in range £7,520 - £12,150 (under review) according to qualifications and experience. Superannuation. Applications, including the names of three referees, should be sent to Professor M I V Jayson, Rheumatic Diseases Centre, Hope Hospital, Salford M6 8HD. (1809)A

POSTDOCTORAL TRAINING IN CANCER BIOLOGY

Postdoctoral Positions are available at the London Regional Cancer Centre (in association with the University of Western Ontario).

Faculty available for training include:
Ann Chambers: Cellular and molecular biology of tumor metastasis in immune deficient and immune-competent experimental hosts.
J. Gregory Cairncross: Cellular and molecular biology of glial differentiation; biology of glial tumors.
Geoff Hammond: Structure function and gene expression of steroid binding proteins; mechanism of steroid entry into target cells.
John F. Harris: Cellular and molecular biology of tumor metastasis in immune-competent hosts; development of mouse and human monoclonal antibody to tumor-associated antigens.
Roger Inch: Sensitivity of multicell spheroids to cytotoxic drugs; magnetic resonance imaging and spectroscopy to monitor tissue damage.
Jim Koropatnick: Gene amplification in mammals, cloning avian and mammalian lectin genes (with H. Ceri, University of Calgary); molecular biology of human hybridoma stability (with Dr. J.F. Harris).

Applications should state the faculty member(s) with whom the candidate wishes to work and should include vitae and references. Inquiries or applications should be sent to:

Dr. Ann Chambers
London Regional Cancer Centre
391 South Street
London, Ontario N6A 4G5
Canada

(NW382)A

The Cancer Center of the University of California, San Diego

Tenure Level Professor

The Cancer Center of the University of California, San Diego, invites applications for a tenured position (full or associate professor) at the Theodore Gildred Facility in a basic science related to cancer (cell or molecular biology, genetics, pharmacology,immunology).

Candidates must have demonstrated independent research productivity and teaching ability. Academic appointment will be in the department most appropriate to the candidate's research and teaching specialties. Salary related to rank and step of appointment within the established salary schedule of the UCSD School of Medicine.

Reply by April 1, 1987 to Stephen B. Howell, M.D., U.C. San Diego Cancer Center, T-012, La Jolla, CA 92093.

The University of California, San Diego, is an Equal Opportunity/ Affirmative Action Employer

(NW374)A

The Brewers' Society

PRINCIPAL OF EDUCATION & TRAINING

£20,000 PLUS

This is a key appointment in the education and training of future technical managers in the brewing industry.
The major challenge for the Principal of Education and Training is to design and manage substantial and complex training programmes aimed at achieving the highest standards of technical competence.
The following qualifications and experience are required:

- Education to university degree standard in a technical discipline
- Experience of brewing technology
- Knowledge of modern training technology
- Successful experience as a manager
- Experience of the design and running of substantial and complex training programmes
- The ability to communicate effectively at all levels

The attractive remuneration package includes a salary of £20,000 + (negotiable) plus a car and the usual fringe benefits provided by the Brewers' Society.
Please write for further details and an application form to: Mr D. Horwood, The Brewers' Society, 42, Portman Square, London W1H 0BB, Telephone (01) 486-4831. (1812)A

University of Oxford

Glaxo Group Research Ltd.

Glaxo

Research Position in Mammalian Cell Molecular Biology

An exciting opportunity has arisen for a post-doctoral researcher to join a large and innovative group with interests in a number of aspects of eukaryotic cell molecular biology. The project will involve analysing the control of DNA replication using viral mini-replicons and developing novel methods for the production of continuous mammalian cell lines expressing foreign proteins. The work will be carried out in the laboratories of Drs S M and A J Kingsman in the Biochemistry Department at Oxford.

High quality researchers with experience in any biochemical/biological discipline are encouraged to apply for this position which is funded by Glaxo Group Research Limited. The position is available immediately but the starting date is negotiable. Funding, in the first instance, is for two years but there are excellent prospects for extension.

The salary will be an appropriate point on the University 1A scale (£8,020 – £11,790).

Applications enclosing a curriculum vitae should be sent directly to Dr S M Kingsman, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU.

General Motors Surgical Research Laboratory
Memorial Sloan-Kettering Cancer Center

RESEARCH SCIENTIST

Ph.D. with 2-5 years experience in areas related to defining cellular and molecular properties of metastatic cancer cells/host factors which might determine organ localization of the cells. Excellent opportunity to develop an independent research program in supporting scientific milieu. The program has been funded.
Send curriculum vitae, bibliography and names of three references to: **Dr. Joseph Fortner, Director, General Motors Surgical Research Laboratory, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue New York, New York 10021 212-794-7466.**

An Equal Opportunity/Affirmative Action Employer

(NW358)A

POPULATION BIOLOGY

The Department of Biology at The University of Chicago announces a search to fill two positions in the area of Population Biology (broadly defined,) at the level of Assistant Professor. We are particularly interested in a research program that combines empirical and theoretical approaches.

Applications are especially invited in the areas of: Molecular Evolution, Plant Population Biology, Microbial Population Biology, Animal Ecology, Community Ecology.

Please send a letter of application, curriculum vitae, reprints and/or recent manuscripts, a statement of research interests, and three letters of reference to: **Search Committee, Department of Biology, The University of Chicago, 940 East 57th Street, Chicago, Illinois 60637**. Deadline for receipt of applications is March 1, 1987.

The University of Chicago is an affirmative action, equal opportunity employer

(NW361)A

LONDON SPORTS MEDICINE INSTITUTE

RESEARCH PHYSIOLOGIST

(preferably Postdoctoral)

The Institute acts as a base for Sports Medicine in London by promoting teaching and research.

Applications are invited from self-motivated suitably qualified persons who wish to pursue a career in Sports Medicine/Science. Applicants must either be medically qualified or possess a good honours degree in applied physiology with at least three years relevant experience and should preferably have a research degree.

The applicant will be responsible for the setting up and running of the physiological testing laboratory and the implementation of physiological research for which he/she may submit proposals.

The post is available immediately and tenable for three years. The starting salary will be related to qualifications and experience.

Applications giving a full c.v. and the names of two referees should be sent to: **Dr Dan Tunstall Pedoe, Medical Director, London Sports Medicine Institute, c/o Medical College of St. Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ.** (1781)A

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

DISTINGUISHED PROFESSORSHIP IN BIOLOGY

The Department of Biology, University of North Carolina at Chapel Hill is pleased to announce the availability of a distinguished professorship to begin July 1988 or possibly sooner. We welcome inquiries from individuals in the fields of Cell Biology, Developmental Biology and Neurobiology. The salary is highly competitive and the position includes a permanent half-time secretary, substantial set-up funds and a custom renovated laboratory. We would appreciate letters of inquiry from potential candidates as well as nominations. All communications will be held in the strictist possible confidence.

For further information, write to: L I Gilbert, Chairman, Department of Biology, Wilson Hall 046A, University of North Carolina, Chapel Hill, NC 27514, or call 919 966 2055.

The University of North Carolina at Chapel Hill is an Equal Opportunity/Affirmative Action Employer

(NW100)A

UNIVERSITY OF ILLINOIS

GENETICIST

The School of Life Sciences, University of Illinois at Urbana-Champaign invites applications for a faculty position at the Associate Professor level. We are seeking a person who is an accomplished geneticist well versed in the modern approaches of molecular and cellular biology. The academic and research record should have a record of sustained significant and innovative research activity in genetic research. A major duty of the successful applicant will be the coordination of the campus-wide graduate genetics program. This administrative responsibility will be balanced by decreased teaching responsibilities. The departmental affiliation of the successful applicant within the School of Life Sciences will depend upon the specific interests of the applicant. Starting date is open. Send resume and names of three references to: **Professor J. Cronan, School of Life Sciences, University of Illinois, 393 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801.** *The University of Illinois is an affirmative action, equal opportunity employer.* (NW359)A

NATIONAL INSTITUTE OF AGRICULTURAL BOTANY

VEGETABLE AND SUGAR BEET PATHOLOGIST

(permanent post)

AND CEREAL PATHOLOGIST

(2 year fixed term contract).

Applications are invited for the above posts in Plant Pathology branch at NIAB headquarters, Cambridge. The successful candidates will join a team evaluating the resistance of new crop varieties to a range of important diseases.

Candidates should have a degree in a biological science with relevant postgraduate training (normally PhD). Appointment will be on grade III (£8,613 - £13,762 pa) with starting salary according to qualifications and experience.

Further particulars and application forms are available from the Personnel Office, NIAB, Huntingdon Road, Cambridge CB3 0LE, tel: (0223) 276381, ext 2234, quoting ref: SV/952/N (Vegetable and Sugar Beet Post) and SV/975/N (Cereal Post). Closing date 6 February. (1808)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

Norfolk Place, London W2 1PG

DEPARTMENT OF CLINICAL PHARMACOLOGY

POSTDOCTORAL RESEARCH FELLOW (NON-CLINICAL)

and

RESEARCH ASSISTANT

Two positions are available immediately in the above department for research in the general field of human vascular biology. The research programme is funded by grants from the British Heart Foundation, Wellcome Trust and industry for studies involving smooth muscle and endothelial cell culture, monoaminergic receptors and vascular tone, vasoactive peptides, second messenger systems and the regulation of intracellular calcium. In additions, techniques are available for the study of vascular reactivity of conduit and resistance vessels *in vitro* and *in vivo*.

The postdoctoral fellowship is for a minimum of two years and the research assistant post for one year in the first instance. Possibilities exist for extension of both positions.

Further information can be obtained from Professor P.S. Sever on 01 725-1256 (direct line). Applications (full c.v. and the names of two referees) must be sent to Assistant Secretary (Personnel) at above address as soon as possible and no later than 28 January 1987. (1777)A

UNITED MEDICAL AND DENTAL SCHOOLS

LABORATORY SCIENTIFIC OFFICER

Experienced Senior Laboratory Scientific Officer required in the Department of Anaesthetics at our Guy's Campus.

Will be expected to play a large part in the Department's research programme, both clinical and basic.

Must be computer literate with experience of, or ability to translate to, IBM PC and XT and Apple IIe. The successful candidate would have the potential to learn new skills and use initiative to work without supervision.

For further information contact Professor A.P. Adams or Dr R.M. Jones on 01-407-7600 extension 2098.

Salary on Whitley scale £8,346—£11,026 plus London Weighting Allowance of £1,113 per annum.

Applications with full c.v. and the names and addresses of two referees, should be sent to the Staffing Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London, SE1 7EH quoting reference: G/ANAES/140. (1784)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

Norfolk Place, London W2 1PG

LECTURER IN ANATOMY

Applications are invited for a Lectureship (to start 1 October 1987) in the Department of Anatomy and Cell Biology. Candidates should have a record of active research in the biomedical sciences and must be able to make a major contribution to one or more of the Department's main teaching areas (Human Anatomy, Cell Biology and Histology, Developmental Biology and Neuroanatomy).

The Department's current research includes Neural Bases of Learning, Alzheimer's disease, Cell Intreractions in Peripheral Nerves, Epithelial Transport Mechanisms, and Advanced Histochemical Methods. No particular research field is specified, but applicants with expertise in immunocytochemical methods and an interest in the setting up of a monoclonal laboratory would be especially welcome.

Further written particulars from Assistant Secretary (Personnel) at above address to whom applications should be sent by 28 February.

Prospective applicants are invited to contact the incoming Head of Department, Dr Anthony Firth at St. George's Hospital Medical School (Tel. 01-672-1255 Ext. 4601) (1776)A

STRANGEWAYS RESEARCH LABORATORY & CELLTECH LIMITED

MOLECULAR BIOLOGIST

POST-DOCTORAL POSITION

This position offers an exciting opportunity for a molecular biologist to broaden an existing collaboration between the Department of Cell Physiology at the Strangeways Research Laboratory in Cambridge and Celltech Limited. The post is for a minimum period of two years and will be based mainly in Cambridge.

Work from the Cell Physiology Department so far has shown that during tissue development and re-modelling metalloproteinases play a key role in the maintenance of the extracellular matrix. These proteinases are normally tightly regulated but in certain disorders, such as arthritis and during tumour invasion, the regulatory mechanisms appear to be altered.

The collaborative programme with Celltech has already resulted in the isolation of cDNAs for these enzymes and for a naturally occurring inhibitor, tissue inhibitor of metalloproteinase (TIMP). The appointed scientist will use cDNA probes to help to extend the biological studies further and should be capable of developing the necessary molecular biology in association with Celltech to use it within a group where existing strengths include cell biology and protein biochemistry.

For further details, contact Dr Andy Docherty at Celltech (0753-36162) or Dr John Reynolds in Cambridge (0223-243231).

The salary offered is £13,000 per annum with normal insurance and superannuation benefits. Applications should be addressed to the Administrative Secretary, Strangeways Research Laboratory, Cambridge CB1 4RN, enclosing a full CV and the names of two referees.

(1821)A

ASSISTANT/ ASSOCIATE PROFESSOR

The Department of Biochemistry of the University of Massachusetts Medical School invites applications for a state-funded, tenure-track position. All areas of biochemistry and molecular biology will be seriously considered, but preference will be extended to those individuals with research interests in either eukaryotic gene regulation and expression or the physical biochemistry of macromolecules. Please send statement of current and future research, curriculum vitae, and names of four references to: **John E. Mole, PhD, Chairman, Search Committee, Department of Biochemistry, University of Massachusetts Medical School, 01605.** *An Affirmative Action/Equal Opportunity Employer.* (NW366)A

The University of Texas Health Science Center at Dallas

Department of Cell Biology

Chairperson

Applications are invited for the position of Professor and Chairperson of the Department of Cell Biology at the University of Texas Health Science Center, Dallas. Candidates must have a distinguished research record and proven leadership and administrative ability. A curriculum vitae and names and addresses of three references should be submitted to the Chairman of the search committee at the following address: Alfred G. Gilman, M.D., Ph.D., Chairman, Department of Pharmacology, University of Texas Health Science Center, Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235.

The University of Texas is an equal opportunity employer.

(NW310)A

SENIOR RESEARCH BIOCHEMIST

The Ferrosan Research Division in Copenhagen, Denmark is seeking candidates for senior research biochemist position in the department of receptor neurochemistry. The Ferrosan Research Division is an innovative organization with a strong commitment to CNS-research. Main areas of research are for the moment the GABA/benzodiazepine receptor complex and excitatory amino acids.

The successful candidate will join a research team dedicated to CNS drug development applying the most modern techniques. Furthermore, the position offers possibilities of undertaking basic research aimed at future drug discoveries in interdisciplinary teams.

The requirements of the position include:

- ★ Ph.D or equivalent in biochemistry or related discipline with an emphasis on mammalian receptor research.
- ★ Minimum of 2 years' post doctoral experience in autoradiographic investigations of CNS-receptors, measurement of energy metabolism in the brain by the deoxyglucose technique and/or CNS/histology.

Please direct applications to:

Dr. T. Honore
Director of biochemistry
A/S FERROSAN RESEARCH DIVISION
5, Sydmarken
DK-2860 Soeborg,
Denmark

Phone +451 692111, ext 242

(W3722)A

Embryologists/ Tissue Culturists

Animal Research Station Cambridge

Two positions — one postdoctoral and one graduate — are immediately available at the Animal Research Station, Cambridge, to join a team working on the *in vitro* maturation and fertilisation of bovine oocytes and culture of resultant embryos. The appointees will undertake both the basic research necessary to establish the techniques and the adaptation of the procedures for the commercial production of embryos for agriculture. This project, based at the Animal Research Station, is being carried out in association with Dr Moor's Department of Molecular Embryology at the AFRC Institute of Animal Physiology & Genetics Research at Babraham.

Candidates should have a strong background in tissue culture; experience in embryology would be an advantage but is not essential. Salaries and benefits, including non-contributory pension, matching the best industrial standards, are offered to stuiably qualified candidates holding a PhD or graduate degree. These appointments are for an initial three-year period.

Applications, including a full curriculum vitae and names and addresses of three referees, should be sent in confidence to Dr C. Polge, F.R.S., ARS Cambridge Ltd., The Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ. (1791)A

University of Toronto, Canada
Department of Geology
Post-Doctoral Research Associate/ Graduate Student Positions
Gold-Quartz Vein/Shear Zone Mineralization in the Canadian Shield

Post-doctoral research associate and graduate student positions (3) are available to work on the geological, structural, petrological and geochemical characteristics and origin of Archean gold-quartz vein/shear zone deposits in the Superior and Slave Provinces. The research programme is well funded by a three-year NSERC strategic grant of C$225,000. Applicants should be interested and have some experience in several of the following fields: Surface and underground structural/lithological mapping, hydrothermal mineralization, igneous geochemistry, isotope geochemistry (Sr-Nd-Pb and light stable) and fluid inclusion analytical techniques. The post-doctoral research associate salary will be in the range C$20,000-C$26,000. Please telephone immediately and/or send CV and two confidential letters of reference to:

Dr. Ed. Spooner, Department of Geology, University of Toronto, Toronto, Ontario, Canada M5S 1A1. Phone (416) 978-3280 (direct); (416) 978-3022 (message); (416) 465-9677 (home). (NW381)A

VETERINARY ANATOMISTS

The Departmentof Anatomy, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, invites applications for tenure track positions in veterinary anatomy at the assistant or associate professor level. Salary and rank will be commensurate with qualifications and experience. Candidates should have a DVM (or equivalent) degree and/or an advanced degree in anatomical science. The position entails maintaining a research program and teaching veterinary gross, microscopic and developmental anatomy in the professional veterinary medical curriculum. Priority will be given to candidates with research interests in cells and tissues, with some preference to studies in the nervous system.

Applicants should send a letter stating professional goals and including a curriculum vitae and the names of 3 references before April 1, 1987.

The résumé should be sent to **Dr. Stephen D. Roper, Chairman, Department of Anatomy, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, 80523.**

Colorado State University is an equal opportunity employer and complies with Title IX requirements. (NW380)A

ST MARY'S HOSPITAL AND MEDICAL SCHOOL

(University of London)
Norfolk Place, London W2 1PG

Departments of Respiratory Medicine and Immunology

POSTDOCTORAL RESEARCH FELLOW

required with experience in the areas of Cellular Immunology, Biochemistry and/or Molecular Biology to join Multidisciplinary Group to investigate Alveolar Macrophage functions in AIDS.

Salary scale £10,865 - £11,275 + £1,297 per annum London Allowance (supported by British Lung Foundation) initially for 2 years to start as soon as possible. Informal enquiries can be made to Dr R Shaw/Dr D M Mitchell, St Mary's Hospital — 01 262 1280.

Applications (3 copies) to the Assistant Secretary (Personnel) at above address to be received by 6 February. (1793)A

THE SCHOOL OF PHARMACY

(University of London)

SENILE DEMENTIA THERAPIES

A 2-year postdoctoral research assistant post is available as part of a multi-disciplinary project. Candidates must be experienced in techniques of animal learning and memory. Salary on the 1A Scale, to start by 1 May 1987.

Applications with two copies of curriculum vitae to: Personnel Secretary, The School of Pharmacy, 29/39 Brunswick Square, WC1N 1AX. Further details can be obtained from Dr S File at the School. (1803)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

MONOCLONAL ANTIBODY DEVELOPMENT

POSTDOCTORAL AND EXPERIENCED POSTGRADUATE SCIENTISTS

required to establish and run a programme for the development of monoclonal antibodies to cellular antigens. Applicants should be qualified to graduate or doctoral level or have suitable experience in a technical position. Practical experience of monoclonal antibody techniques would be a considerable advantage. The posts will be funded for up to 3 years. Salary commensurate with qualifications and experience.

Please send full curriculum vitae to Professor T.J. Chambers, Department of Histopathology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. (1800)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF HAEMATOLOGICAL MEDICINE
SCHOOL OF CLINICAL MEDICINE, HILLS ROAD

POSTDOCTORAL RESEARCH ASSISTANT

Molecular Abnormalities of Coagulation

Applications are invited for a 3-year MRC funded position to develop techniques for the investigation of molecular mechanisms of coagulation and their abnormalities. Experience in protein chemistry or molecular biology is desirable but the position could also suit someone (not necessarily postdoctoral) with broad experience in coagulation biochemistry.

Enquiries and applications to Professor R.W. Carrell, Haematology Department, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. Telephone Cambridge 336788. (1783)A

UNIVERSITY COLLEGE, LONDON

DEPARTMENT OF BIOCHEMISTRY

TECHNICAL ASSISTANT

(Grade 3)

required in the laboratory of Dr P.W. Piper. The post is funded by a 3-year SERC project grant for yeast molecular biology and is available immediately. Salary in the range £7,333—£8,405 inc. LW. Applications giving details of age, qualifications, experience and two referees should be sent to Dr P.W. Piper, Department of Biochemistry, University College London, Gower Street, London WC1E 6BT to arrive no later than 31 January 1987. (1807)A

THE UNIVERSITY OF MANCHESTER

POSTDOCTORAL RESEARCH ASSOCIATE IN RADIO ASTRONOMY

Applications invited for the above post in the Nuffield Radio Astronomy Laboratories, Jodrell Bank.

Research Associates are expected to carry out their own research and assist with existing research activities. The main facilities are the 76m mk 1A telescope (used for single dish work, MERLIN and VLBI) and the optical studies of active galaxies, radio spectral line studies of galaxies, cosmic masers, pulsars, stars, and the microwave background. Appointment tenable from October 1st, 1987 initially for a period of 2 years with a possibility of extension up to a maximum of 3 years. Salary on Research 1A Scale (£8,020—£12,780 per annum) (under review) depending on age and experience. Superannuation. Enquiries to: The Director, Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire. SK11 9DL (1772)A

MOLECULAR BIOLOGY GROUP LEADER

Agouron Pharmaceuticals is a rapidly growing company committed to the exploitation of protein structure in the design of novel therapeutic molecules. Agouron's scientific strategy relies upon strong interdisciplinary interactions between molecular biology, protein crystallography, and organic chemistry. Our state-of-the-art facilities occupy a seaside location in the vigorous scientific community of La Jolla, California.

We seek a senior scientist (not less than five years of postdoctoral experience) with a broad background and an abiding interest in the cloning, characterization and expression of prokaryotic and eukaryotic genes as group leader in molecular biology. Attractive compensation package includes stock options.

Send *curriculum vitae* and references in strict confidence to: Reference MB

(NW387)A

Agouron
Pharmaceuticals, Inc.
505 Coast Boulevard South
La Jolla, California 92037

DIRECTOR

Monterey Bay Research Institute

The Board of Trustees of the Monterey Bay Aquarium Foundation requests nominations and applications for an outstanding *Director* for the newly founded *Monterey Bay Research Institute* to be located in Monterey, California, independent of but closely associated with the Monterey Bay Aquarium. The Institute will be housed in new facilities which the Director will help to design and will have a guaranteed operating budget of $2 million per year.

The goals of the Institute will be to create a premier program of marine research based upon the unique opportunities offered by the diverse marine environments of Monterey Bay, its submarine canyon, and the contiguous ocean waters. The Institute will emphasize interdisciplinary approaches centered on biology, but also involving geological, physical and chemical oceanographic studies. It will foster research that focuses on variability through time as well as in space, on time scales ranging from diurnal to decadal. It will encourage advances in research through support and development of new technology and instrumentation. The Institute will be committed to education, through University affiliations, through training of post-doctoral scholars, and through association with the public education mission of the Monterey Bay Aquarium.

The Director must be a distinguished scientist of international reputation with broad interests related to life in the sea and its relations to physical, chemical, and geological processes. The Director should be a "modern naturalist" with experience in contemporary experimental science and field work as well as an appreciation for the development of technologies for furthering such research.

The Director will be charged with the development and management of the Institute in addition to continuing his/her own research program. The Director will have the assistance of an advisory board consisting of eminent scientists and engineers. The appointment as Director is for five years, and will be renewable or continued as a senior research position in the Institute.

Send requests for further information, nominations, or letters of interest with curriculum vitae and names of four references to:

Search Committee
MONTEREY BAY AQUARIUM FOUNDATION
886 Cannery Row
Monterey, CA 93940

Nominations and applications will be accepted until March 1, 1987.

(NW379)A

The national centre of blood transfusion in France

one of the largest of its kind in Europe,
is looking for a

CELL BIOLOGIST

to work in the development of cell cultures.
You will be responsible for optimizing and developing selected cell lines to ensure production by genetically engineered cell lines and you will be involved in a strategic project conducted in close collaboration with several french and international teams.
Ideally, you will have Ph D or equivalent qualifications, plus two years experience in eucaryotes cell culture technology.
You should also be perfectly bilingual in english and french. Future prospects are excellent as you'd expect in such a significant specialist position.

Applications with curriculum vitae and the names and addresses of two referees (who will not be approached without your prior consent) should be sent to : Elisabeth VERGER, BP 100 ; 91943 Les ULIS CEDEX. FRANCE. Further information can be obtained from H. VAN DE POL. 1.69.07.78.71. Closing date 8 th February.

(W3721)A

media system

CNTS — FONDATION

RESEARCH SCIENTISTS

Investigate Genelabs, a young, well financed and promising biotechnology company entering the diagnostic and therapeutic health care fields. Key opportunities exist for the following professionals:

Molecular Biologist: Strong research capabilities in library construction & screening using both phage and plasmics; background in gene expression. PhD & 3 yrs. exp.

Cell Biologist: Strong background in areas of mammalian tissue culture, virology, somatic cell hybrids & interest in molecular biological characteristics of cells. PhD with 3-5 yrs. exp.

Molecular Immunologist: Exceptional background in lymphokines, functional immune assays, hybridoma research & T-cell cloning techniques. MD, PhD or equiv. with 3-5 yrs. relevant exp.

Protein Chemist/Immunochemist: PhD or equiv. with 3-5 yrs. exp. in the following immunochemical techniques: 1 & 2 D SDS-Page; Western blotting; MAb purification; enzyme & fluorochrome conjugation of MAbs & other proteins; immunoaffinity purification of cellular & secreted proteins. Exp. with automated protein sequencing and peptide synthesis useful.

Molecular Biologist: PhD with min. 3 yrs. focusing on eukaryotic expression systems including mammalian expression systems using SV-40, retroviral & other vectors.

*Opportunities also for qualified **Research Assistants & Associates**.

Please send CV and names of 3 references in confidence to: **GENELABS INCORPORATED,** 871 Industrial Road, Bldg. J, San Carlos, CA 94070. An equal opportunity employer. Principals only, please.

GENELABS
INCORPORATED

(NW357)A

THE BIOCHEMICAL SOCIETY

requires a

DEPUTY EDITORIAL MANAGER

for its Editorial Office in central London. The successful applicant will be required to carry out subeditorial work on the full range of the Society's journals and books, taking specific responsibility for certain of them, and will act as the Deputy to the Editorial Manager. He or she will have a degree (preferably a higher degree) in biochemistry or a related subject, several years' experience in scientific publishing, an interest in the application of information technology to the area, and the ability to supervise the work of others. Starting salary will be approx. £13,500.

Applications, including full curriculum vitae and the names of two referees, to the **Editorial Manager, The Biochemical Society, 7 Warwick Court, High Holborn, LONDON WC1R 5DP, by 9 February 1987.** (1820)A

UNIVERSITY OF LEEDS

POSTDOCTORAL IMMUNOLOGIST

An industry assisted post to develop a model to investigate Acne vulgaris in association with the University Departments of Immunology, Microbiology and Dermatology. Experience in Immunology essential. Some experience in microbiological and/or histological procedures would be an advantage. Applications in writing to **Prof. G. Gowland, Dept. of Immunology, The General Infirmary, Leeds, LS1 3EX** by January 21st 1987. Informal enquiries: Prof G. Gowland. 0532-432799 Ext. 3540 or Dr K.T. Holland 0532-441199 Ext. 233. (1749)A

POSTDOCTORAL RESEARCH ASSOCIATE

position available to study regulation of contractile protein function in skeletal and cardiac muscle, using combined biochemical and mechanical techniques with skinned fibre preparations. Individuals with appropriate background in physiology or biochemistry should send curriculum vitae and, names of three references to:

Franklin Fuchs, Ph.D., Department of Physiology, University of Pittsburgh, School of Medicine, 645A Scaife Hall, Pittsburgh, PA 15261. (NW378)A

FACULTY POSITION AVAILABLE

The University of California, Berkeley, has an opening, effective July 1, 1987, in the Department of Mechanical Engineering for a tenured position at the Associate or full Professor level, in the area of applied aerodynamics. Candidates should have a doctoral degree, a strong academic background in aerodynamics, and some background in the application of aerodynamics to design the performance of flight vehicles.

Send biography and the names of four references by March 31, 1987 to: **Applied Aerodynamics Search Committee, c/o Chairman, Department of Mechanical Engineering, University of California, Berkeley, California 94720. The University of California is an Equal Opportunity, Affirmative Action Employer.** (NW383)A

MOLECULAR BIOLOGIST

Opportunity with rapidly growing probe technology company for Molecular Biologist (PhD Molecular Biology/Biochemistry). Candidate must be thoroughly experienced in all areas of molecular cloning including library construction, chromosome walking, subcloning, northern and southern blotting. Competitive salary and benefits. Send c.v., references and salary history in confidence to:

oncor®

ONCOR, Molecular Biologist Position, Box 870, Gaithersburg, MD 20877. (NW384)A

UNIVERSITY OF BIRMINGHAM

FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY

BIOCHEMICAL TOXICOLOGY (B23)

Applications are invited for a postdoctoral research fellowship supported by the MRC, to study the activation of pro-mutagens by animal and human hepatocytes and the effect of anti-oxidants. Applicants should have a relevant background in Biochemical Toxicology. Tenable for up to twenty-months.

Tenable from as soon as possible on the Research Fellow 1A scale £8,020 to £12,780 plus superannuation. For further particulars phone 021-472 1301 ext 2559, quoting reference number given above. Three copies of application, including a c.v. and the names of three referees should be made by 31 January to the Assistant Registrar, Faculty of Science, University of Birmingham, P.O. Box 363, Birmingham B15 2TT. An Equal Opportunities Employer. (1779)A

THE UNIVERSITY OF THE WEST INDIES, ST AUGUSTINE, TRINIDAD

ADVERTISEMENT FOR VETERINARY BIOCHEMIST

Applications are invited for a post of Senior Lecturer/Lecturer in Biochemistry, School of Veterinary Medicine. Required basic training in Biochemistry and/or the DVM or equivalent. Relevant teaching and research and/or service experience in Chemical Pathology desirable.

Annual Salary Range: DVM qualified - Senior Lecturer: TT$84,084 - $101,856; Lecturer: TT$65,412 - $82,584. Other qualification - Senior Lecturer: TT$69,180 - $90,168; Lecturer: TT$54,708 - $77,604. Pension, Passages, Housing. Three copies of detailed application naming three referees to the Registrar, UWI. Further particulars available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (W3729)A

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF ASTRONOMY

POSTDOCTORAL RESEARCH ASSOCIATE IN THEORETICAL ASTRONOMY

MONTE CARLO SIMULATIONS OF THE OORT CLOUD

Applications are invited for a 2-year PDRA funded by the SERC to work with Dr M.E. Bailey and Professor F.D. Kahn on cometary dynamics. The project will involve calculations of cometary orbits and statistical studies of their evolution in the Oort Cloud and from the Oort Cloud to the planetary system. The primary aim is to explore models containing a massive inner core. Initial salary on RA1A Scale up to £9,495 p.a. (under review).

Superannuation. Applicants should send c.v., copies of recent papers, and arrange for three referees to write to: Dr M.E. Bailey, Department of Astronomy, the University, Manchester M13 9PL (from whom further details are available). Closing date for applications and referees February 13th, 1987. (1770)A

FACULTY: PHYSIOLOGIST

The Department of Biology and Geosciences, West Texas State University, Canyon, TX is seeking PhD applicants for a tenure-track assistant professor position in physiology. The individual will be expected to teach anatomy and physiology and advanced physiology. Before 10 March 1987, please send resume, transcripts, three letters of reference, and statement of research to: **Dr F.C. Killebrew, Chairman of Search Committee, Dept. of Biology & Geosciences, West Texas State University, P.O. Box 808, W.T. Station, Canyon, Texas 79016-0808.**

WTSU is an equal opportunity/affirmative action employer. (NW369)A

ASSISTANT PROFESSOR

A tenure-track position is available in the Department of Physiology. With active programs in cell and membrane biology and biophysics we are seeking an individual in the area of CYTOSKELETON AND CELL MOTILITY. This area is construed in the broadest sense to include diverse disciplinary approaches and systems. Applications and three letters of recommendation should be sent to: **Dr Richard D. Berlin, Professor and Head, Department of Physiology, University of Connecticut Health Center, Farmington, CT 06032.**

An Affirmative Action/Equal Opportunity Employer, M/W/H. (NW364)A

THE UNIVERSITY COLLEGE OF WALES, ABERYSTWYTH

WELSH PLANT BREEDING STATION

(Grant-aided by the Agricultural and Food Research Council)

HIGHER SCIENTIFIC OFFICER (BAND I)/ SENIOR SCIENTIFIC OFFICER

Applications are invited for a Rhizobium Geneticist/Biochemist in the newly formed Nitrogen Fixation Group of the Plants and Soils Division of the Welsh Plant Breeding Station, which will be regarded as the centre for applied work on biological nitrogen fixation within the Agricultural and Food Research Service.

Qualifications: First or Upper Second Class Honours Degree in microbiology or biochemistry with a microbiological bias. At least 4 years relevant post qualifying experience is required for SSO, 2 years for HSO. Experience in physiology/biochemisty and/or practical experience of *Rhizobium* genetics would be advantageous.

Pay Scales:
(SSO) £10,558 — £13,762
(HSO) £8,405 — £11,323

Non-contributory pension scheme but all male employees contribute 1½% to widow's pension benefits.

Annual leave: 22 days plus 10½ Public and Privilege holidays per annum.

Written requests for further particulars and application forms should be made to **The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed, SY23 3EB, to whom completed application forms should be returned. Closing date for applications is 6 February 1987. (Quote reference 86/18).**

The WPBS is an equal opportunities employer. (1813)A

ANALYTICAL CHEMIST required for routine work (nmr, vpc, ir, hplc etc.). Suit fresh graduate with approx. 1 year's industrial experience. Write giving experience & phone no. to Maybridge Chemical Co. Ltd., Trevillett, Tintagel, Cornwall PL34 0HW. (1780)A

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international
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— EVERY WEEK

MEDICAL RESEARCH COUNCIL

LABORATORY OF MOLECULAR BIOLOGY

POSTDOCTORAL POSITION
(Ref: PNAC/2274)

Applications are invited from postdoctoral scientists to participate in a project on structural studies of membrane proteins including mitochondrial ATPase and ion transporters. Experience with protein chemistry and/or crystallography would be an advantage, but relevant experience in other areas of molecular biology or biochemistry will be taken into consideration.

Further information on the project is available from Dr J E Walker (Cambridge (0223) 248011).

The post is available for 3 years and the salary will be in the range £9,495 — £15,700 pa.

RESEARCH ASSISTANT
(Ref: PNAC/RA)

Applications are invited from graduate biochemists or molecular biologists to work on the assembly, crystallisation and structure of membrane proteins including mitochondrial ATPase and ion transporters. Experience in protein and/or nucleic acid chemistry could be an advantage.

Further information on the project is available from Dr J E Walker Cambridge (0223) 248011.

Salary will be in the range of £8,020 — £12,280 pa.

Applications — including a full CV and the names of two professional referees — should be sent by 5 January, 1987 quoting references PNAC/2274 or PNAC/RA to:— The Personnel Officer, Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH. (1810)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF PLANT SCIENCE

POSTDOCTORAL RESEARCH FELLOW

to join a group working with Dr I. Alexander on the biology of mycorrhizas. The project concerns structural and physiological aspects of ageing and death of ectomycorrhizas and will involve transmission electron microscopy and radiotracer studies of carbon and phosphorus movement. Candidates should hold a PhD in a relevant discipline. This is an NERC-funded post tenable for 3 years from 14 April 1987.

Starting salary £8,020 per annum, the 1st point on the Range 1A Scale for Research and Analogous Staff (under review).

Further particulars and application forms from The Personnel Section, The University, Regent Walk, Aberdeen AB9 1FX, to whom applications (2 copies) including a letter of application and c.v. should be returned by 6 February 1987 (Ref WS/014). (1775)A

LUDWIG INSTITUTE FOR CANCER RESEARCH
Melbourne Tumour Biology Branch

MOLECULAR BIOLOGISTS

The Lugwig Institute has been established in Melbourne to conduct a programme of laboratory and clinical research in conjunction with the Royal Melbourne Hospital, The Walter and Eliza Hall Institute and the University of Melbourne. Our research programme is directed towards understanding the control of cell growth in normal and neoplastic tissues.

Two postdoctoral research fellow positions are available in the molecular biology laboratory.

REF: 1/87
A Postdoctoral/Research Fellow

is required to study the role played by members of the SRC-family of tyrosine protein kinases in the growth and differentiation of haemopoietic cells. Candidates should have a sound background in molecular biology and experience in protein biochemistry/immunology would be an advantage. For further information, contact Dr. Ashley R. Dunn.

REF: 2/87
A Postdoctoral/Research Fellow Position

is available to study the interactions of the protein encoded by the MTB oncogene with nuclear components of Haemopoietic cells. Candidates should have a background in molecular biology, and experience in DNA-protein interactions and chromatin structure would be an advantage. For further information, contact Dr Thomas J. Gonda.

Salary will be in accordance with qualifications and experience within the range A$27,000 — $37,000. In the first instance, both appointments are for 2 – 3 years duration. Appointees are eligible to join an excellent superannuation scheme. Travel and removal expenses, including initial accommodation costs, will be covered by the institute. Travel to scientific conferences and relevant laboratories overseas is encouraged.

Applications, which should include curriculum vitae and the names of at least two referees, should be forwarded, before April 10, 1987, to:

Dr. A.W. Burgess, Director, Melbourne Tumour Biology Branch, Ludwig Institute for Cancer Research, PO Royal Melbourne Hospital, Vic. 3050, Australia. (W3735)A

UNIVERSITY OF CAMBRIDGE

Department of Applied Biology

A vacancy exists for a

POSTDOCTORAL RESEARCH ASSISTANT

To join a collaborative project studying the ecological effects of pesticides in intensive cereal production.

The post funded by MAFF is for one year starting on 1 March or 1 April 1987. The research programme will be to develop ecological studies of shrews and small rodents with particular emphasis on their feeding habits and interaction with invertebrates. A knowledge of invertebrates and experience in their identification will be an advantage. A full driving licence is essential. Salary will be in the range £6,110 - £10,375 pa.

Applications, naming two referees should be received by Friday 30 January 1987. Further details may be obtained from the Administration Secretary, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX. Tel: 0223 334400. (1789)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

Department of Histopathology

PART-TIME RESEARCH TECHNICIAN

Applications are invited for a experienced and qualified histopathology technician to take part in a project involving the preparation of resin-embedded undecalcified bone sections. Opportunities may exist also to participate on an exchange basis in the routine work of the Department of Histopathology.

Part-time post for 18½ hours per week at the appropriate point according to age and experience on the salary scale for medical laboratory scientific officers. The post is tenable for 1 year initially. Experience in bone histopathology is not essential.

Application form from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, 01 672 1255 ext 5481 (telephone answering service). Closing date 5 February 1987. Quote reference 1/87. (1801)A

RESEARCH MANAGER

YEAST GENETICS

Research Manager, Yeast Genetics to conduct original independent research involving industrial strains of aneuploid and polyploid saccharomyces, including definition of genomes, compilation of genomic mapping, single chromosome transfers, genetic modification by recombinant and mutagenic techniques, and administrative responsibilities associated with the establishment of an industrial yeast genetics laboratory. Position is with manufacturer of alcoholic beverages and food products in St. Louis, Missouri. Qualified applicants must have PhD in Applied Microbiology and have demonstrated expertise in original research involving industrial strains of aneuploid and polyploid saccharomyces. Starting salary $50,000 per year plus company benefit plan for 40 hour week. Send resume accompanied by details of research expertise to:

Ms Kathryn Gooch, ID #035634, JOB SERVICE, 505 Washington, St. Louis, Missouri 63101.

An Equal Opportunity Employer. (NW363)A

BIOTECHNOLOGY GROUP, UKAEA HARWELL LABORATORY

and

MEDICAL RESEARCH COUNCIL, RADIOBIOLOGY UNIT, CHILTON

CELL SURFACE MARKERS

Applications are invited for a

SHORT-TERM NON-CLINICAL SCIENTIFIC POST

(tenable for 3 years)

to study the appearance of molecular markers on the surface of oncogenically transformed mammalian cells. Applicants should be acquainted with modern techniques of biochemical analysis and should preferably have some experience of the culture of mammalian cells, the isolation of cell membranes and/or the immunology of cell surfaces. The successful candidate will be based in the MRC Radiobiology Unit but will be attached to the staff of the adjacent Biotechnology Group of Harwell Laboratory where the majority of the studies will be performed. Both centres have substantial programmes in related areas which range from fundamental studies on oncogenesis to technical service to industry and there is considerable scope for collaborative studies.

Renumeration will be at an appropriate point on the scales for university non-clinical academic staff and application forms may be obtained from the Personnel Officer, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD with whom applications including a full CV and the names of two proffesional referees should be lodged. Closing date: 16th February 1987.

The Council is an Equal Opportunity Employer. (1811)A

UNIVERSITY COLLEGE LONDON

POSTGRADUATE OR POSTDOCTORAL POSITION IN STREPTOMYCETE MOLECULAR BIOLOGY

Applications are invited for a 3-year post funded by the SERC and a consortium of companies. The project involves a detailed study of Streptomycete plasmid transfer and replication and includes DNA sequencing, RNA transcript analysis and construction of various Streptomycete plasmid derivatives to study transfer and replication *in vivo*. The post is available immediately. Starting salary on the appropriate University scale depending on age, qualifications and experience. Applicants with experience in microbiology, genetics or biochemistry should send a curriculum vitae and names of two referees to either Dr J.M. Ward (Biochemistry Dept.) or Dr H. Richards (Botany & Microbiology Dept.), University College London, Gower Street, London WC1E 6BT. Informal enquiries to Dr Ward (ext 2242) or Dr Richards (ext 2934), tel. (01) 387 7050. (1806)A

SOUTH MANCHESTER HEALTH AUTHORITY PATERSON INSTITUTE FOR CANCER RESEARCH CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE

Manchester M20 9BX, UK

A post-doctoral appointment is available to work on projects concerned with early mechanisms in chemically induced cancer. The post is financed as part of a Cancer Research Campaign programme and is for three years initially.

Applicants should preferably have a background in biochemistry. The appointee will work principally in collaboration with Dr R Saffhill to study the interaction of higher alkylating agents with DNA in vitro and in vivo. Techniques invlolve the use of hplc, radioimmunoassays and monoclonal antibodies.

Applications could also be considered from suitably qualified Senior Research Officers, in which case the MRC Research Officers salary scale would apply.

The use of these techniques to detect environmental exposure to carcinogenic alkylating agents will be an important part of this project.

Salary according to age and experience on MRC Scale Grade II.

Applications, including cv and names of referees should be made to Dr PJ O'Connor, Head, Division of Chemical Carcinogenesis, at the above address as soon as possible. (1823)A

UNIVERSITY OF GLASGOW

DEPARTMENT OF MEDICINE

Royal Infirmary

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant which will be attached to the Renal Unit of the Department of Medicine at the Royal Infirmary, Glasgow. The post is funded for three years and is suitable for a graduate who wishes to acquire or who has just acquired a PhD. The work will examine the physiological aspects of the glomerulus and their effect on disease. Experience in Physiology, Immunology or Biochemistry would be an advantage.

Salary will be within range 1B or 1A of the salary scales for Research and Analogous Staff, with placement according to age, qualifications and experience.

Enquiries to Dr J M Boulton Jones, Renal Unit, Royal Infirmary, Glasgow. Applications to Professor A C Kennedy, University Department of Medicine, Royal Infirmary, Glasgow. (1822)A

UNIVERSITY OF LIVERPOOL

Department of Veterinary Pathology

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Applications are invited from virologists for the above post to assist with research on turkey rhinotracheitis. The appointment is for two years in the first instance and will be based at the Veterinary Field Station, Neston, Wirral.

Expertise in immunological techniques would be an advantage. Initial salary within the range £8,020 - £9,000 per annum (under review).

Applications, together with the names of three referees, should be received not later than 29 January 1987, by The Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/334/N. (1790)A

UNIVERSITY OF PENNSYLVANIA

CELLULAR PHYSIOLOGY

Postdoctoral positions to carry out research on structure and mechanism of the sodium pump protein. Two major areas: (I) Transport and biochemical studies in reconstituted red cell ghosts; (II) Chemical and photochemical modification of purified renal Na, K-ATPase. Techniques employed include, tracer kinetic studies, photoaffinity labeling, use of caged ATP, proteolytic analysis, etc. Suitable for recent doctoral graduates in Biochemistry, Physiology or related discipline. Contact: **Dr Jack H. Kaplan, Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104-6085.** *Equal opportunity/affirmative action employer.* (NW353)A

ASTON UNIVERSITY

Diabetes Research

CONTRACT RESEARCH ASSISTANT/OFFICER IN BIOCHEMISTRY/ ENDOCRINOLOGY

An interesting research position exists for a well qualified graduate (Research Assistant) or recently qualified postdoctoral scientist (Research Officer) to join a research group, in the department of molecular sciences, investigating hormonal aspects of diabetes mellitus. The appointee will engage in a study of the interaction of biguanides and insulin in the control of glucose uptake and metabolism.

The post is for one year in the first instance with possible renewal for a further one or two years. Commencing salary will be within the range £7,055 to £9,495 per annum (presently under review), depending upon qualifications, age and experience.

Application forms and further particulars may be obtained from the personnel officer (academic staff), quoting ref: 867516, Aston University, Aston Triangle, Birmingham B4 7ET. Tel: 021 359 8070 (24-hour answerphone). Closing date for the receipt of applications is 13th February 1987. (1817)A

SEMINARS & SYMPOSIA

LEUKAEMIA RESEARCH FUND

International Research Symposium on

"CHROMOSOMES AND GENES IN LEUKAEMIA"

at the Royal College of Physicians, London NW1

on MONDAY 27th APRIL 1987

Registration fee (including lunch): £15

For programme details and registration form apply to:

LEUKAEMIA RESEARCH FUND, 43 Great Ormond Street, London WC1N 3JJ (tel: 01 405 0101)

(1796)M

continued on page 21

Royal Society Research Professorships
Locke Research Fellowship
Mr and Mrs John Jaffé Donation Research Fellowship

The Council of the Royal Society invites applications for appointments tenable from 1 October 1987:

1. Three Royal Society Research Professorships, for research in any branch of science (including mathematics, engineering and technology, agriculture and medicine).
2. A Locke Research Fellowship for research in physiology or pharmacology.
3. A Mr and Mrs John Jaffé Donation Research Fellowship for research in the biological sciences.

Appointments are subject to the Society's General Regulations governing Royal Society Research Professorships (1) and Research Appointments (2) and (3).

All candidates must have a Ph.D. or equivalent research experience. Candidates for 1 (which will be supported from the Society's Parliamentary Grant-in-aid) must be citizens of the United Kingdom or the Commonwealth and hold the appointment in any appropriate university or research institution in the United Kingdom. There are no restrictions on nationality or place of tenure for 2 or 3.

Salaries (under review) and conditions of tenure:

1. Salary within the normal professorial range. Tenure for five years in the first instance, renewable thereafter, usually until age 65.
2. Salary scale £11,917 x £513 to £14,482 per annum. Tenure for three years in the first instance, possible renewal for two years.
3. Salary scale £8,675 x £491 to £9,166 x £381 to £9,547 x £491 to £11,020 per annum. Tenure for three years in the first instance, possible renewal for two years.

For all appointments superannuation benefits will be provided and there will be some provision for research expenses. The point of entry on the relevant salary scale will be determined by Council.

Preferred age limits (on 1 October 1987):
1. None. 2. Preferably under 33. 3. Preferably under 26.

Method of application:
For 1: Intending applicants should write for further details (there is no application form).
For 2: Apply (by letter) for application form and further details (*stating whether for Locke or Jaffé appointment*).

Address:
The Executive Secretary (UMAM), The Royal Society,
6 Carlton House Terrace, London, SW1Y 5AG.

Closing date: 16 February 1987.

November 1986

THE ROYAL SOCIETY

Royal Society
University Research Fellowships
(established 1983)

The Council of the Royal Society invites applications for 20 appointments to be supported from the Society's Parliamentary Grant-in-aid and tenable from 1 October 1987 as Royal Society 1983 University Research Fellowships.

All applicants must have a Ph.D. or equivalent research experience; they must be at least 26 but should not have passed their 33rd birthdays by 1 October 1987. In an exceptional case, however, an award may be made to an applicant who has passed his/her 33rd birthday by this date but who has outstanding ability for independent scientific research and who is able to demonstrate that there are special reasons why he/she should still be eligible for a fixed-term research fellowship. Applicants must be British Citizens (of the United Kingdom) and must hold the appointments in departments of science (including agriculture, medicine, mathematics, engineering and technology) in universities in the United Kingdom (these appointments are *not* tenable in polytechnics). Persons already holding substantive posts in universities in the United Kingdom will not normally be considered.

Appointments, which will be subject to the Society's General Regulations governing Royal Society Research Appointments, will be for up to five years with the possibility of renewal for two successive periods of up to three and two years. Salary will be on the University Lecturers' scale for non-clinical academic and related staff with entry point related to age and London Allowance where appropriate. A basic grant of £1050 per annum will be provided for research expenses.

The primary criterion for selection will be the scientific merit of the individual applicant based on his or her record so far and on future potential. Account will be taken of the facilities and intellectual environment being offered by the prospective host department.

Applicants who were unsuccessful in previous years should not be deterred from applying in 1987 if they are eligible to do so. They should, however, make a fresh application on a new application form and arrange for updated statements from referees to be provided.

Please send written request for application form and further details.

Address: The Executive Secretary (UMAM),
The Royal Society,
6 Carlton House Terrace,
London, SW1Y 5AG.

Closing date: 16 February 1987

November 1986
(1489)E

FELLOWSHIPS continued

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

SPRING 1987 AWARDS

Next deadline: 16 February 1987

EMBO long term postdoctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but depending on a review of progress, they may be renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 16 February 1987. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 27 April 1987.

Application forms and further details may be obtained from Dr J Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F. R. Germany. Inquiries should be accompanied by a self-addressed adhesive label.

(W3595)E

THE HATFIELD POLYTECHNIC

POSTDOCTORAL FELLOWSHIP IN MOLECULAR BIOLOGY

Applications are invited from suitably qualified biologists to work on *one* of the following projects:

(i) The mechanism of chloroplast DNA replication in higher plants (Dr S. Boffey 07072-79547).

(ii) The isolation and characterisation of enzyme inhibitors with pharmacological activity from algae (Dr J. Walker 07072-79546).

The post is funded by the NAB Research Initiative and is tenable for two years (salary: Researcher B £8,595—£9,573 plus £282 local allowance).

Applications (no forms) giving full details of relevant experience and the names of two academic referees should be addressed to the Staffing Office, The Hatfield Polytechnic, PO Box 109, Hatfield, Herts, AL10 9AB. Please quote reference 221. (1795)E

GRADUATE FELLOWSHIPS

Center For The Study Of Evolution And The Origin of Life

UCLA

CSEOL invites applications for three first-year predoctoral fellowships for 1987 – 88. Stipends are each $9,000 for 9 months with no teaching or other duties. Fellows are included in a select group of former Fellows and other graduate students invited to interact with Center faculty in a weekly dinner/seminar/discussion series. Applicants should be planning to carry out thesis research under the supervision of a CSEOL faculty member and in a subject area relating to evolution, broadly construed — of the solar system, of Earth, of the environment, of life, of humans. Areas of particular interest for 1987 – 88 include (1) the origin of life; (2) Precambrian biotic-environmental evolution; (3) biochemical phylogeny; and (4) the interface between molecular biology and the known fossil record. Faculty are members of the UCLA Departments of Anthropology, Astronomy, Atmospheric Sciences, Biology, Chemistry & Biochemistry, Computer Science, Earth & Space Sciences, Geography, and Microbiology; the School of Medicine (Anatomy; Microbiology & Immunology; Physiology; Biobehavioural Sciences); and the School of Public Health.

Fellowship applicants must apply by February 15, 1987, both to an appropriate academic department and to CSEOL.

For application forms and a list of CSEOL members, contact:

CSEOL Fellows Committee
Geology Building, Room 5687
University of California, Los Angeles
Los Angeles, CA 90024
Telephone: (213) 825-1170 (NW385)E

MILLIKAN POSTDOCTORAL FELLOWSHIPS IN EXPERIMENTAL PHYSICS AND ASTROPHYSICS

Caltech's Division of Physics, Mathematics and Astronomy offers several Millikan Fellowships for postdoctoral study in experimental physics or astrophysics at the California Institute of Technology. These Fellowships will have a two- or three-year duration, beginning in Fall 1987, and will carry a stipend of $29,000 per year. In addition, the Fellow will have access to a research-expense fund of $2,000 per year.

The Millikan Fellowship Program has been established to offer junior scientists the opportunity of pursuing new and innovative experimental research. It is expected that this research will require the support and use of facilities of one of the established experimental groups at Caltech. Currently, Caltech has groups working in:

- **Astronomical Detector Development**
- **Gravitational Physics**
- **High Energy Astrophysics**
- **High Energy Physics**
- **Infrared and Submillimeter Astronomy**
- **Low Energy Particle Physics**
- **Low Temperature Physics**
- **Neurobiology**
- **Nuclear Physics**
- **Radio Astronomy**
- **Surface Physics**

The application should indicate the group with which the candidate would most likely be associated and discuss particular areas of interest.

Candidates may either apply directly, or they may be nominated by colleagues or professors. Materials in support of an application or nomination should be sent to Professor E. C. Stone, Chairman, Division of Physics, Mathematics and Astronomy, 103-33, California Institute of Technology, Pasadena, CA 91125. These materials should include curriculum vitae, bibliography of publications and preprints of manuscripts not yet published, and a description of the anticipated research program. The candidate or nominator is requested to ensure that at least three letters of recommendation be sent directly to Caltech.

ALL MATERIALS SHOULD ARRIVE AT CALTECH BEFORE MARCH 1, 1987

Fellowship candidates will automatically be considered for other available postdoctoral positions at Caltech in their fields of interest.

Caltech is an Affirmative Action/Equal Opportunity Employer. Women/minorities are encouraged to apply. (NW365)E

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

School of Earth Sciences

RESEARCH FELLOWSHIP GEOLOGY/GEOPHYSICS

Applications are invited for a Research Fellowship tenable in the School of Earth Sciences. Applicants should possess a PhD degree or have had equivalent relevant research experience. The position is available for one year from 1 October 1987 and may be renewed for a further 12 months. Preference will be given to applicants in the general areas of marine geology and marine geophysics although the School also has research interests in oceanography, meteorology and hydrology. The School has unusually good support staff and facilities for experimental activities and numerical techniques. Preliminary enquiries may be directed to the Dean of the School, Professor G W Lennon, with whom details of specific research activities and facilities within the School may be clarified.

The appointment will be made according to qualifications and experience within the salary range: $A24,013 - $27,507. An appointee holding a PhD degree will not be appointed below the third level of the salary scale ($A25,414). An appointee from interstate or overseas will be eligible for assistance with travel and removal expenses.

Applications, including full curriculum vitae, academic record and publication list, a brief outline of research interests and names and addresses of three referees, should be lodged, in duplicate, with the Registrar, The Flinders University of South Australia, Bedford Park, South Australia 5042, by 30 January 1987, or as soon as possible thereafter.

(W3728)E

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nature — the professionals' choice

GRANTS & SCHOLARSHIPS

THE ROYAL SOCIETY THE RUTHERFORD SCHOLARSHIP

This award, for graduates of exceptional promise and ability who are under 26 years of age on 1 May 1987, is for research in any branch of the natural sciences, with a preference for experimental physics if there are candidates of similar merit. It will be tenable for three years, from 1 October 1987 or such other date as may be arranged, in some part of the British Commonwealth other than that in which the applicant graduated. The value, adjusted to meet the circumstances, will be £4,610 per annum on appointment if held in the United Kingdom, with allowances for travel, contribution to university fees, etc. It will not normally be awarded to a graduate who has already held a senior research award.

Applications should be made as follows:

From university graduates *in the United Kingdom*: before 15 February 1987 on forms obtainable from the Executive Secretary (UMAM), The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG.

From university graduates *outside the United Kingdom*: through their universities to the Royal Commission for the Exhibition of 1851 on forms of application for the Commissioners' Science Research Scholarship to be obtained from the registrars or administrative authorities of their universities. (1771)H

BRITISH FOUNDATION FOR AGE RESEARCH RESEARCH GRANTS

The Foundation for Age Research aims to improve the quality of life for the old by funding medical and scientific research into the diseases and disabilities associated with the ageing process.

Applications are invited for: (a) Research projects; (b) Senior Registrar's Equipment. Grants for Research (up to £10,000).

Closing date for receipt of completed applications: 1st March 1987.

Application forms available from: The Administrator, Foundation for Age Research, 49 Queen Victoria Street, London EC4N 4SA (A4 sized 24p stamped addressed envelope please). (1792)A

nature

— the world's most prestigious weekly journal of science

STUDENTSHIPS

BRITISH MUSEUM (NATURAL HISTORY) POSTGRADUATE RESEARCH STUDENTSHIPS 1987

Two studentships will be available, tenable for 3 years, at the British Museum (Natural History) commencing October 1987.

Applications are invited for the following three research projects, the two to receive studentships will be selected following interviews. Arrangements will be made with an appropriate university for joint supervision of studentships and higher degree registration.

DEPARTMENT OF ENTOMOLOGY

1. The classification and biology of dry forest *Spilochalcis* species in Central America.
2. An experimental and morphometric investigation of the *Pulvinaria vitis* complex in Europe.

DEPARTMENT OF BOTANY

1. Palynology and Systematics of Central American Acanthaceae.

A wide range of scientific facilities is available and the student will have full access to the Museum's extensive libraries.

Eligibility: Applicants must be ordinarily resident in Great Britain, and have a first or upper second class honours degree: a student graduating in 1987 can be offered an award subject to his/her degree result reaching this level.

Students interested in applying should write to the Secretary (Postgraduate Studentships), British Museum (Natural History), Cromwell Road, London SW7 5BD for application forms, conditions of the awards and further information on the projects.

Closing date for receipt of completed application forms 2 March 1987. (1804)F

SEMINARS & SYMPOSIA continued

REPRODUCTION AND DISEASE IN CAPTIVE AND WILD ANIMALS

19 & 20 FEBRUARY 1987

A Symposium to be held by the Zoological Society of London and The Wellcome Trust. Details from: Assistant Editor, Zoological Society of London, Regent's Park, London NW1 4RY: Tel 01-722-3333. (1814)M

First CBT (Center for Biotechnology) Minisymposium on:

NUCLEAR ONCOPROTEINS: ROLES IN INDUCTION AND MAINTENANCE OF NEOPLASIA

STOCKHOLM

June 12-13, 1987

Organizing Committee:

Rolf Ohlsson Rune Toftgard Georg Klein

Programme:

- ☐ Control of gene expression
- ☐ Hormone receptors
- ☐ Molecular basis for oncoprotein functions
- ☐ Cell cycle progression
- ☐ Cellular maturation
- ☐ Cellular transformation
- ☐ Transgenic mice

Invited participants:

J. Adams *(Viktoria)*
F. Alt *(New York)*
M. Cole *(Princeton)*
R. Eisenman *(Seattle)*
T. Graf *(Heidelberg)*
P. Leder *(Boston)*
R. Muller *(Heidelberg)*
K. Nilsson *(Uppsala)*
U. Rapp *(Frederick)*
R. Roeder *(New York)*
D. Stehelin *(Lille)*
R. Watt *(Philadelphia)*
E. Ziff *(New York)*
G. Akusjarvi *(Uppsala)*
P. Chambon *(Strasbourg)*
B. DeChrombrugghe *(Bethesda)*
T. Gonda *(Viktoria)*
M. Groudine *(Seattle)*
K. Marcu *(New York)*
A. Nienhuis *(Bethesda)*
M. Oren *(Rehovot)*
E.P. Reddy *(Philadelphia)*
D. Stehelin *(Lille)*
R. Watt *(Philadelphia)*
I.M. Verma *(San Diego)*

Applications:

A letter of application, containing a brief outline of research interests and ambitions, should be sent to the address below before March 15, 1987. The total number of participants will be limited to 125.

Rolf Ohlsson, Center for Biotechnology, Karolinska Institute, Huddinge University Hospital F82, S-141 86 Huddinge, Sweden

(W3727)M

5th Cell Surface Research Fund Meeting

London 9 & 10 April 1987

EXOCYTOSIS: Post-receptor events in secretory cells

This meeting, held jointly with the Membrane Group of the Biochemical Society, will take place on Thursday and Friday, 9 and 10 April at St George's Hospital Medical School, London SW17. Thursday will be devoted to general mechanisms, including the biophysics of membrane fusion and membrane traffic; Friday will be devoted to specific systems, including mast cells, chromaffin cells, neutrophils, endocrine and exocrine cells, neurones and T lymphocytes. Speakers will include the following:

P.F. Baker (London)
D. Bar-Sagi (Cold Spring Harbor)
R.D. Burgoyne (Liverpool)
J. Davy (Dundee)
R.L. Dormer (Cardiff)
J.M. Fernandez (Philadelphia)
R.C. Foreman (London)
M.J. Geisow (Nottingham)
B.D.Gomperts (London)
P. Henkart (Bethesda)
R.W. Holz (Ann Arbor)
E.K. Matthews (Cambridge)
J.J. Nordmann (Strasbourg)
R. Penner (Gottingen)
T. Pozzan (Padova)
T Schafer (Basel)
M.J. Whitaker (London)
C.B. Wollheim (Geneva)
J. Zimmerberg (Bethesda)

Time will be set aside on each day for poster sessions, and intending participants are invited to submit a poster: please send titles to the organiser at the address below.

Registration fee of £50 includes coffee, buffet lunch and tea on each day; dinner will be available each evening for £20 (inclusive of wine). Accommodation with breakfast at nearby Halls of Residence is £180 (inclusive of registration and all meals) from Wednesday night until Saturday morning; participants may stay until Sunday if desired. Overnight accommodation (bed and breakfast only) £30. Students should apply for special rates. **Please contact Mrs V. Marvell, Department of Biochemistry, St George's Hospital Medical School, London SW17 0RE; 01-767 6910 for registration forms and further details as soon as possible.** (1815)M

LECTURES

FIDIA RESEARCH FOUNDATION PRESENTS THE SECOND CAMILLO GOLGI AWARD LECTURE

"Coexistence of Multiple Neuronal Messengers: New Aspects on Chemical Transmission" honoree and speaker Tomas Hokfelt, M.D., Professor of Histology, Karolinska Institute on 5 February 1987 at 4.00 pm at Georgetown University Medical Center, 3900 Reservoir Road, N.W., Washington, D.C.

For further information, contact: Fidia Research Foundation, 1775 K Street, N.W., Suite 801, Washington, D.C. 20006; Telephone: 202 857 4277. Telex: 4970490.

(NW367)K

CONFERENCES & COURSES

MSc in Medical Microbiology

(University of London)

London School of Hygiene and Tropical Medicine and the Joint Medical School of University College

Advanced academic, practical and clinical training in all branches of medical microbiology for one year is provided by a full-time course by the Department of Medical Microbiology and other Departments of the School with clinical teaching by the staff of University College and the Middlesex Hospital Medical School and the Teaching Hospitals of the Schools and the Bloomsbury District Health Authority.

Specialised options for 10-12 weeks in either advanced medical bacteriology, virology, clinical microbiology, immunology, medical mycology, medical parasitology or molecular biology are available.

The MSc course is suitable for candidates qualified in medicine, dentistry, veterinary medicine, science, pharmacy and to others with approved qualifications and experience.

Successful candidates are eligible for exemption from the examination for the Primary MRCPath if other College requirements are satisfied.

Direct applications to the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, Great Britain. Application deadline: 1 May 1987.

Diploma in Medical Microbiology

A six months course of academic and practical training in selected topics,leading to the School's Diploma.

Direct applications to the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, Great Britain. Application deadline: 1 May 1987. (1389)C

NATO ADVANCED STUDIES INSTITUTE

TARGETING OF DRUGS:

ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS

20 June – 1 July 1987,
Cape Sounion Beach, Greece

LECTURES WILL COVER: (a) Microanatomy and physiology of the blood, RES, lymphatic and immune systems, vascular endothelium, basement membranes and membrane receptors; (b) Interaction of the biological milieus with drug carrier systems eg neoglycoproteins, monoclonal antibodies, polymers, microparticles and liposomes: Implications for passive and receptor-mediated drug targeting of conventional and recombinant drugs (eg for cancer and antimicrobial treatment, vaccines, etc); (c) relevant methodology; (d) industrial and clinical aspects.

LECTURERS AND TUTORS WILL INCLUDE: A.C. Allison (Palo Alto), G. Ashwell (Bethesda), A.D. Bangham (Cambridge), L. Chedid (Paris), S.S. Davis (Nottingham), I.J. Fidler (Houston), G. Gregoriadis (London), J.G. Hall (Sutton), F.K. Jansen (Montpellier), V. Kolb-Bachofen (Düsseldorf), L. Liotta (Bethesda), N. McIntyre (London), M. Monsigny (Orleans), G. Palade (New Haven), G. Poste (Philadelphia), G. Scherphof (Groningen), K. Sikora (London), P. Thorpe (London), E. Wisse (Brussels).

Send application (there is no special form) with a brief c.v. and scientific interests to the ASI Director: Dr Gregory Gregoriadis, Medical Research Council Group, Academic Department of Medicine, Royal Free Hospital School of Medicine, London NW3 2QG, England. (Tel 01-794 0500 ext 3999). Upon acceptance (by 30 March 1987) a fee of UK sterling £350 will be required to cover registration and accommodation (sharing) half-board. Limited to 100 persons. A number of applicants will be considered for financial support.

Facilities for POSTERS and selected SHORT COMMUNICATIONS. ORGANISING COMMITTEE: **A.C. Allison, S.S. Davis, G. Gregoriadis (ASI Director and Chairman), G. Poste (ASI Co-Director) and K. Sikora.**

ASI co-sponsored by SMITH KLINE FRENCH LABORATORIES

(1819)C

UNIVERSITY OF SURREY

DEPARTMENT OF BIOCHEMISTRY

MSc in TOXICOLOGY

Applications are invited for a postgraduate course leading to an MSc degree in toxicology. The course, which may be attended either full-time or part-time, is organised by the Department of Biochemistry, University of Surrey, in collaboration with the MRC Toxicology Unit, Carshalton, the British Industrial Biological Research Association, Carshalton, Shell Toxicology (Tunstall) Laboratory, Sittingbourne and the Robens Institute of Industrial and Environmental Health and Safety, Guildford. During the course instruction is given in these collaborative laboratories as well as at other major toxicological institutions. Entrance requirements are as for a PhD and a limited number of grants may be available for suitable candidates.

The course is designed to provide medical, veterinary and science graduates, with an understanding and appreciation of the many disciplines involved in toxicology. Particular emphasis is placed on aspects related to the biochemical mechanisms of chemically induced toxicity, and the assessment of toxicological hazard in the manufacture, use and disposal of new chemical compounds, pesticides and medicines. The course provides a broad biological education and is a suitable introduction for those intending to undertake research on the interaction of chemicals with biological systems.

There are numerous interesting opportunities for scientists, educated initially in different disciplines, who have acquired additional knowledge of toxicology. These may be in industry, government regulatory bodies, or research institutes with good career prospects in both laboratory work and administration.

Further information and application forms to be returned as soon as possible but by no later than either 2nd May 1987 (British and EEC applicants) or 1st June 1987 (other applicants), can be obtained from: **Dr R.S. Jones, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, UK.** (1774)C

continued on pages 23 & 24

CONGRESS

Entering the X century of the University of Bologna

International Congress CANCER METASTASIS Biological and Biochemical Mechanisms and Clinical Aspects

Bologna (Italy), May 13-15, 1987

Organized by: UNIVERSITY OF BOLOGNA, FONDAZIONE INTERNAZIONALE MENARINI. *With the sponsorship of:* ASSOCIAZIONE ITALIANA PER LA RICERCA SUL CANCRO, LEGA ITALIANA PER LA LOTTA CONTRO I TUMORI, METASTASIS RESEARCH SOCIETY

The meeting will cover the most recent biological, biochemical and clinical aspects of cancer metastasis. The programme will include lectures by invited speakers and poster sessions.

Deadline for abstracts: February 28, 1987

Invited participants:

A. Aaronson, USA; A. Albini, Italy; P. Alexander, UK; P. Altevogt, FRG; L. Barbara, Italy; W. Birchmeier, FRG; M. Bracke, Belgium; M.A. Brunelli, Italy; L. Chieco Bianchi, Italy; F.J. Cleton, Nederland; M.I. Colnaghi, Italy; P.M. Comoglio, Italy; C.H. Damsky, USA; P. De Baetselier, Belgium; C. De Giovanni, Italy; G. Della Porta, Italy; M.B. Donati, Italy; S. Eccles, UK; L. Eisenbach, Israel; M. Feldman, Israel; S. Ferrone, USA; I.J. Fidler, USA; G. Fossati, Italy; P. Frost, USA; A. Fulton, USA; L.T. Furcht, USA; S. Garattini, Italy; S. Garbisa, Italy; L. Gennari, Italy; R. Giavazzi, Italy; T. Giraldi, Italy; G.I. Gozzetti, Italy; P. Gullino, Italy; I. Hart, UK; K. Hellmann, UK; R. Herberman, USA; K. Karre, Sweden; R. Kerbel, Canada; H. Kobayashi, Japan; L. Kopper, Hungary; H. Koprowski, USA; L. Larizza, Italy; L. Liotta, USA; P.-L. Lollini, Italy; A.M. Mancini, Italy; A. Mantovani, Italy; D. Marrano, Italy; L. Milas, USA; G. Nicolson, USA; F. Pannuti, Italy; G. Parmiani, Italy; R. Pozzatti, USA; M.F. Poupon, France; G. Prodi, Italy; A. Raz, Israel; S. Rosenberg, USA; A. Sacchi, Italy; L. Santi, Italy; V. Schirrmacher, FRG; S. Segal, Israel; B. Sloane, USA; M. Sobel, USA; M. Spina, Italy; F. Spreafico, Italy; E.V. Sugarbaker, USA; P. Sugarbaker, USA; J. Talmadge, USA; D. Tarin, UK; J.P. Thiery, France; A. Vaheri, Finland; I. Vlodavsky, Israel; L. Weiss, USA; D. Woolley, UK.

SCIENTIFIC SECRETARIAT: Istituto di Cancerologia dell'Università di Bologna, Viale Filopanti, 22 1-40126 Bologna (Italy). *ORGANIZING SECRETARIAT*: Fondazione Internazionale Menarini, Piazza del Carmine, 4 1-20121 Milano, (Italy). Tel. (392) 874932–804739

(W3726)Q

ANNOUNCING . . .

THE FOURTH ANNUAL CONGRESS FOR

AUTOMATION, SCALE-UP and the ECONOMICS OF BIOLOGICAL PROCESS ENGINEERING

MARCH 4 - 5, 1987

MOSCONE CENTER, SAN FRANCISCO

A one and one half day meeting in conjunction with:
THE SEVENTH ANNUAL DNA/HYBRIDOMA CONGRESS EXPOSITION

ORGANIZED BY SCHERAGO ASSOCIATES, INC.
in conjunction with GENETIC ENGINEERING NEWS

CHAIRMAN: Gerald L. Hawk, PhD, Zymark Corporation

TOPICS:

Cell Propagation • Fermentation • Cell Optimization • Cell Characterization • Cell/Tissue Culture • Process Monitoring and Control • Automated Immunoassay • Purification • Biosensors • Human Genome Project • Drug Delivery Systems • Respect to GMP & GLP • Regulation Economic Issues • Automated DNA Sequencing • Automated Plasmid DNA Purification

For further information concerning registration and accommodations, contact:

Convention Dept.
Scherago Associates, Inc.
1515 Broadway
New York, NY 10036
(212) 730-1050

(NW354)Q

9th INTERNATIONAL CLAY CONFERENCE 1989 AIPEA

Association Internationale pour l'Etude des Argiles

Strasbourg, France, August 28th - September 2nd 1989

Secretary General: **Dr Hélène Paquet**
Institut de Géologie
1, rue Blessig, 67084 Strasbourg, France

(W3725)C

COURSES & CONFERENCES continued

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION PRACTICAL COURSE

"GENETIC MANIPULATION OF *STREPTOMYCES*"

JOHN INNES INSTITUTE, NORWICH, ENGLAND

18–31 July 1987

The course will include gene cloning, protoplast fusion, mating, phages and plasmids. Instructors: M.J. Bibb, C.J. Bruton, M.J. Buttner, K.F. Chater, D.A. Hopwood, H.M. Kieser, T. Kieser, R.E. Melton and J. White.

Further particulars and application forms from Professor D.A. Hopwood, John Innes Institute, Colney Lane, Norwich NR4 7UH, England. (Deadline for applications 1 April 1987.) (1499)C

IMPERIAL CANCER RESEARCH FUND

DNA TUMOUR VIRUS MEETING 1987

The Imperial Cancer Research Fund is sponsoring a meeting on DNA Tumour Viruses to be held in Churchill College, England from 27th July to 1st August 1987 (5 days). The subject of the meeting will be the molecular biology of small DNA tumour viruses, particularly polyoma virus, SV40 and adenoviruses. It will replace for this year the annual Cold Spring Harbour DNA tumour virus meeting.

The meeting is open to all interested research workers. The scientific programme will include oral presentations and posters. The cost for registration, accommodation, food and the abstract book will be £245.

Anyone interested in attending the meeting should apply immediately for further details to: **Miss Patricia Latter, ICRF DNA Tumour Virus Meeting Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A3 PX, England.** (1788)C

APPOINTMENTS WANTED

advertise your qualities and qualifications through the most influential science weekly in the world at a special reduced cost of ***50p per word, minimum 16 words****. (Personal Box Numbers £2).*
Advertisements must be pre-paid and sent to: Nature Classified (Appts Wtd), 4 Little Essex Street, London WC2R 3LF. (890)B

MISCELLANEOUS

Research Vessel for Economical World-Wide Charter

A 55′6″ × 19′ research vessel with a range of 6,000 miles at eight knots for charter party of six.

Experienced crew, paravene stabilized and extensive electronics.

Spice Island Traders — One Boston Place, Boston, MA 02108 (617) 723–1955 (NW440)J

WORKSHOPS

EMBO WORKSHOP

Crystalline Cell Envelope Layers in Microorganisms

(structure, chemistry, biosynthesis, morphogenesis, genetics, function and biotechnological application) at

Universität für Bodenkultur, Vienna, AUSTRIA
31st August – 4th September 1987

Deadline for applications:
28th FEBRUARY 1987

Details: **Prof. Uwe B. Sleytr, Zentrum für Ultrastrukturforschung, Universität für Bodenkultur, Gregor Mendel Straße 33, A-1180 Vienna/Austria.**

(W3719)V

continued on page 2

Carolina Workshops on GENERAL DNA TECHNOLOGY

Sequencing & Mutagenesis

A general DNA techniques workshop in which shotgun cloning into M13 vectors, dideoxy sequencing of the random subclones, computer generation of sequence overlaps and in vitro mutagenesis by the gapped-duplex approach are highlighted. Students may bring their own DNA or be provided with DNA for sequencing. Tuition is $1400.

Instructors

Alan Bankier, MRC, *Cambridge, England*

Ann H. Erickson, *UNC-CH*

Wilfried Kramer, Max-Planck *Martinsried, West Germany*

May 29 - June 14, 1987

Cloning cDNA into Expression Vectors

A general DNA techniques workshop in which synthesis of cDNA and cloning into lambda gt-11 are highlighted. Students may bring their own or be provided with mRNA and antibody to screen for expression of the desired cDNA. Tuition is $1400.

Instructors

Marshall Hall Edgell, *UNC-CH*

Richard Ogden, Agouron Institute, *San Diego*

J. Gregor Sutcliffe, Scripps Clinic, *La Jolla*

July 6 - 22, 1987

The Program in Molecular Biology & Biotechnology
University of North Carolina at Chapel Hill

CAROLINA WORKSHOPS are a series of intensive hands-on laboratory courses designed to teach cutting edge methods in DNA technology. The techniques include basic manipulations such as isolation of insert and vector DNA, ligations, electrophoretic analysis and elution from gels, blotting techniques, preparation and labeling of probes, and identification of cloned DNA. Each course provides experience in the basic techniques which serve as a solid, basic background in DNA technology. Each individual course has an identifiable theme and emphasizes in addition to the general techniques, specialized techniques such as DNA sequencing, in vitro mutagenesis, cDNA synthesis, and/or expression vectors. Students are given the opportunity to learn the techniques while also using their own starting materials or may choose to be provided with starting materials.

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will be held at Kensington Town Hall, London, July 2 & 3 1987

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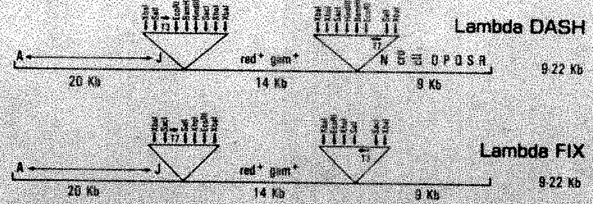

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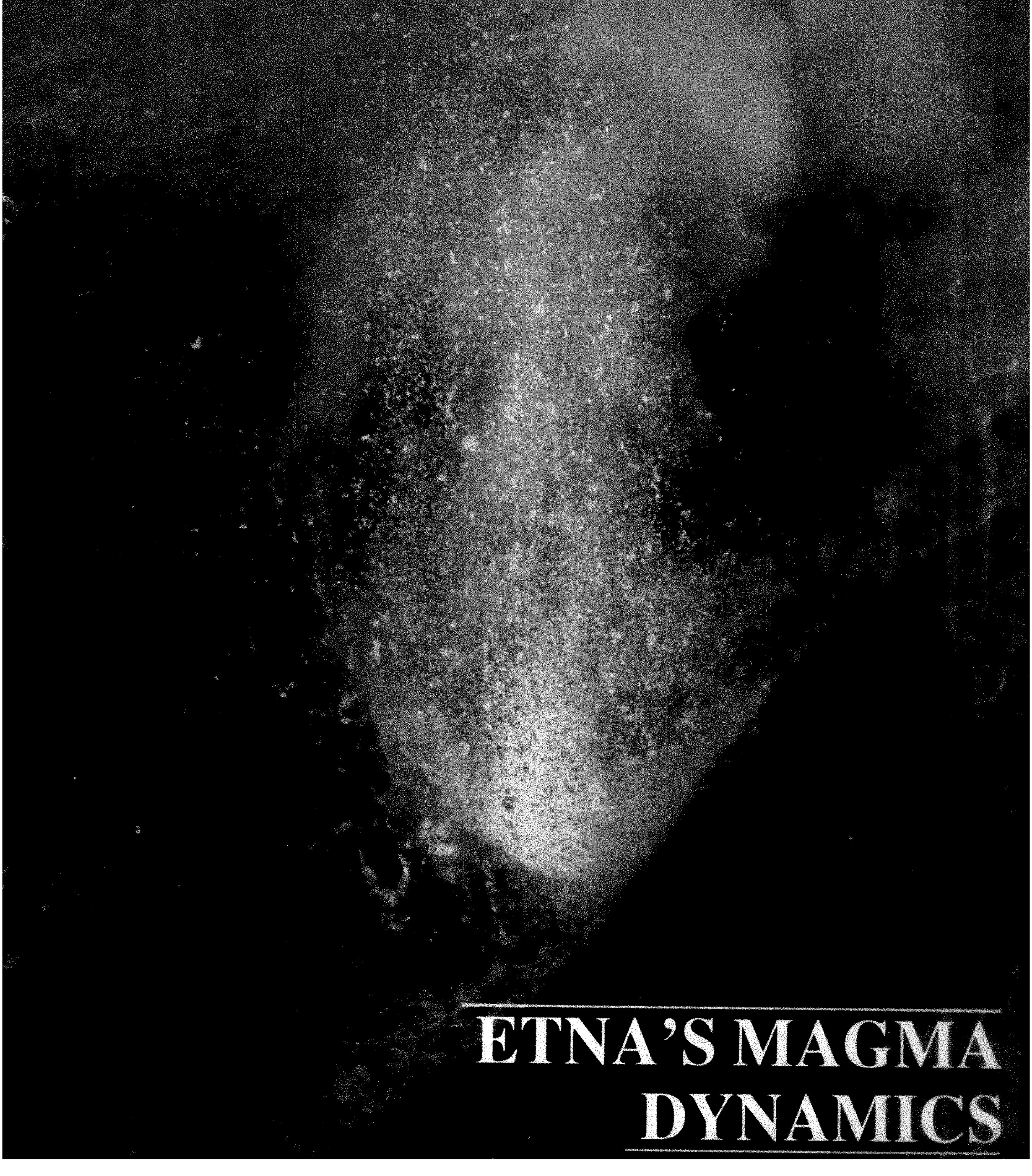

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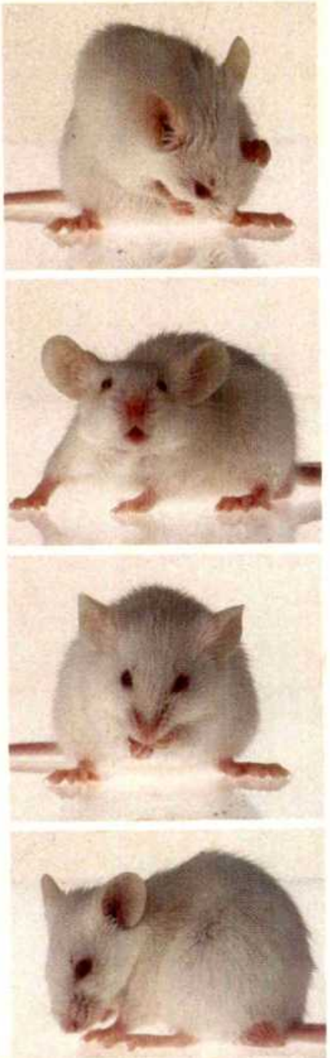

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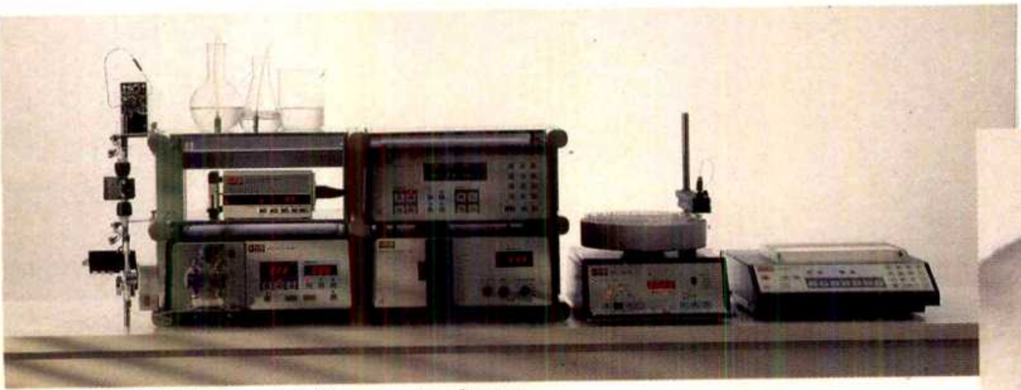

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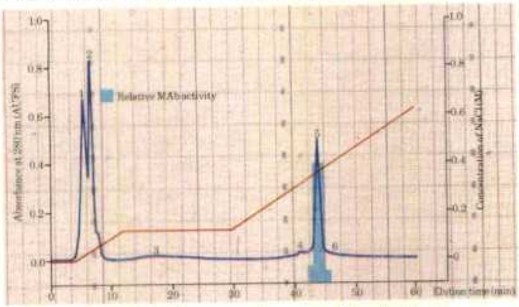
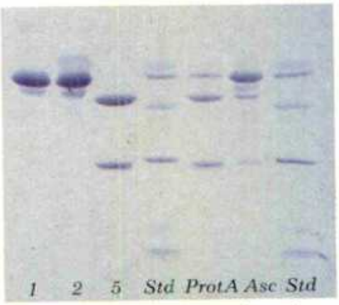


Purification of IgG$_1$ monoclonal antibody from mouse ascites fluid.



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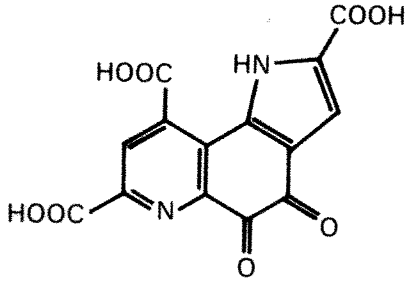

Methoxatin, a **P**yrrolo**Q**uinoline**Q**uinone, is the cofactor of a new class of bacterial dehydrogenases, called quinoproteins, which are neither flavin nor nicotinamide dependent[1]. Many dehydrogenases contain this novel prosthetic group: methanol dehydrogenases[2] and methylamine dehydrogenase[3] in methylotrophs, ethanol dehydrogenase in Pseudomonas[4] or in acetic acid bacteria[5], D-glucose dehydrogenase of acetic acid bacteria[6], Pseudomonas[5,7], Acinetobacter calcoaceticus[1], Escherichia coli[8] and other bacteria[9] and glycerol dehydrogenase of acetic acid bacteria[10]. More recently, a polyethylene glycol dehydrogenase of a mixed culture of Flavobacterium and Pseudomonas[11], and a poly(vinyl alcohol) dehydrogenase[12] of Pseudomonas were found.

Most interestingly, it was established that PQQ occurs in mammalian enzymes too: a bovine serum amine oxidase has covalently bound PQQ[13] and a choline dehydrogenase of mammalian liver was shown to be a quinoprotein as well[14]. This finding suggests the possibility that PQQ may be required as a cofactor for other mammalian enzymes too. It may thus be a dietary requirement (vitamin)[15].

PQQ was found to be a growth factor[16] or a growth-stimulating factor[17] for microorganisms. The lag phase in microbial growth is markedly reduced if PQQ-containing culture broth of Escherichia coli is added[18].

PQQ itself can foster oxidation reactions: e.g. the catalytic oxidation of amines to aldehydes and ketones[19] and of thiols to disulfides[20] and the oxidative decarboxylation of amino acids[21].

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64682

Methoxatin, PQQ
(4,5-Dihydro-4,5-dioxo-1H-pyrrolo[2.3-f]quinoline-2,7,9-tricarboxylic acid)
$C_{14}H_6N_2O_8$ Mr 330.21 [72909-34-3]
puriss. ~99%(HPLC); H_2O 10-15%
10 mg sFr. 55.– / 50 mg sFr. 219.—

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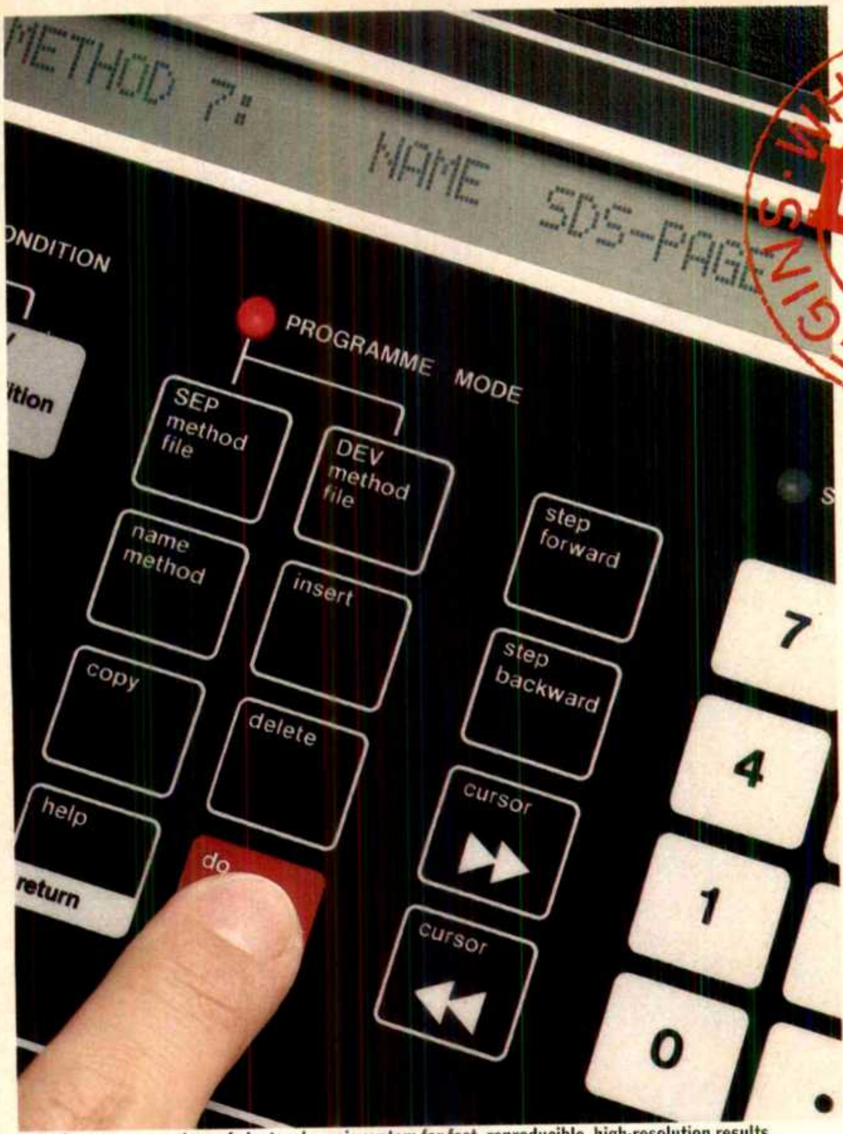

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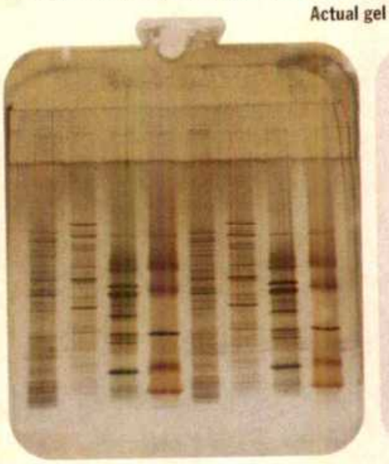

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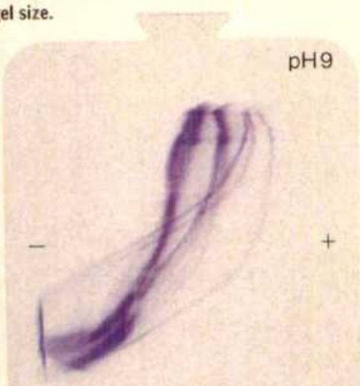

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nature
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Mount Etna in eruption, 29 November 1986, showing a lava fountain more than 200 m high on Etna's east flank. For the past 15 years, Mount Etna has shown a high eruptive activity (on average one flank eruption a year, with persistent summit activity) and provides a unique opportunity for volcanological and geochemical studies. See pages 607 and 573. Photo: J C Tanguy.

THIS WEEK

■ Unearthly crystals

. . . page 601

The laboratory formation of metastable cubic ice crystals adds credence to the belief that they account for Scheiner's halo, sometimes seen around the Sun or Moon.

■ Deep-sea clock

. . . page 616

An internal biological clock controls annual blooming of the microorganism causing 'paralytic shellfish poisoning'.

■ Yesterday's weather

. . . page 587

Fossilized beetle remains reveal the pattern of changes in the British climate during the past 22,000 years.

■ Pesticides in fog

. . . page 602

Concentrated pesticides are present in the water droplets of fog samples from Maryland and California.

■ Luminous arcs

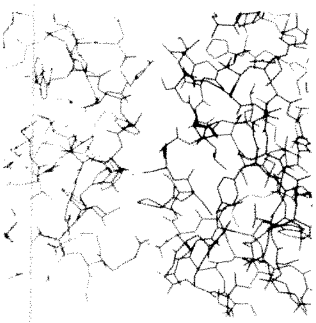
. . . page 572

This giant galactic structure is real, but what is it?

■ Protein mobility

. . . pages 575 and 643

X-ray diffuse scattering can provide details about intramolecular mobility of protein molecules (lysozyme, above), with an important bearing on biological activity.



■ Oncogenes and disease

. . . pages 631 & 635

The *c-abl* proto-oncogene is activated in a novel way in some forms of acute lymphoblastic leukaemia.

. . . page 574

Mammalian oncogenes may be implicated not only in tumour induction but also in formation of the atherosclerotic plaque that causes coronary heart disease.

■ Disappearing neurons

. . . pages 578 & 617

A set of brain cells appearing transiently in the developing mammalian brain have properties like those of mature neurons and may set the patterns for later development.

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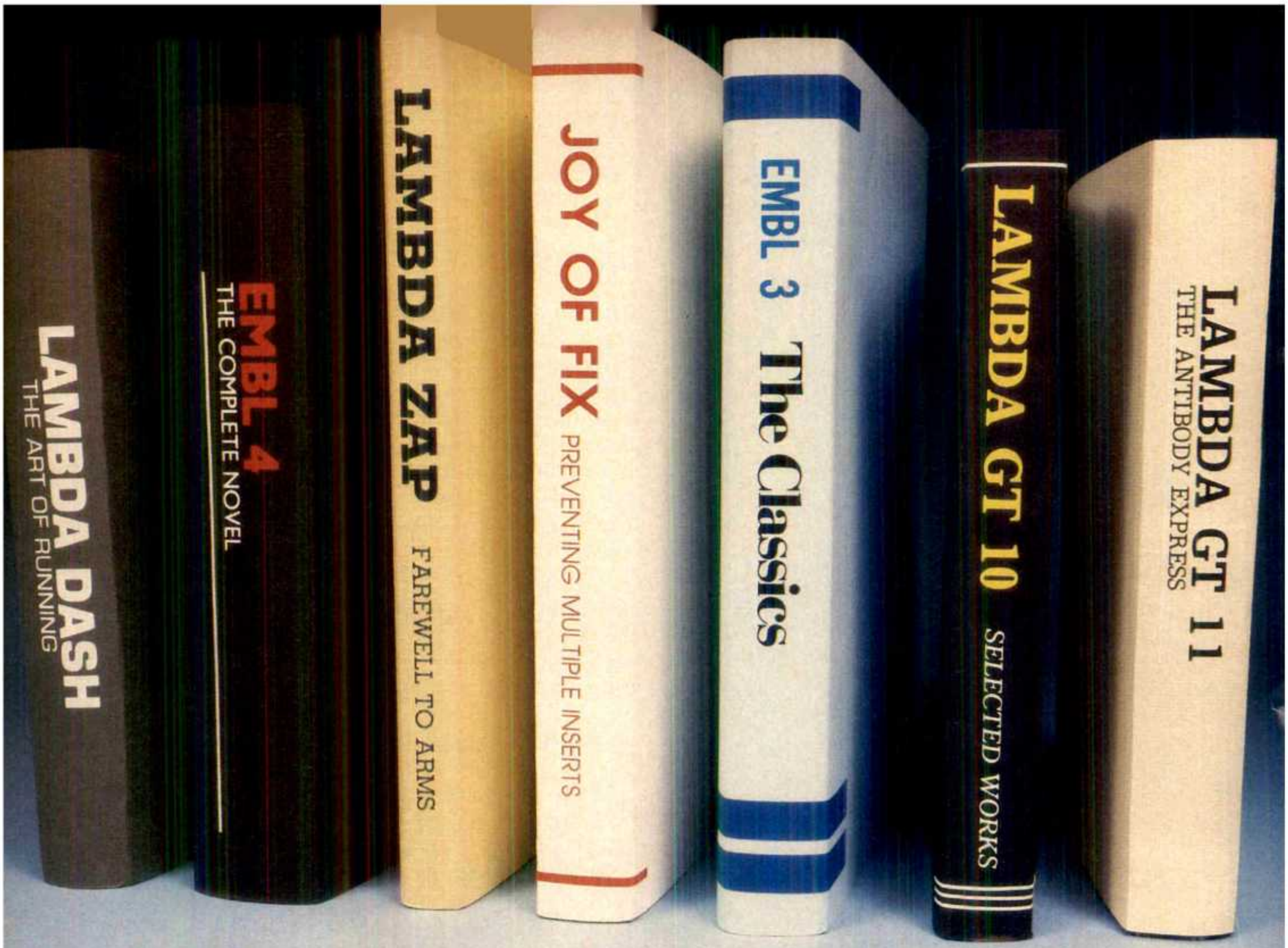

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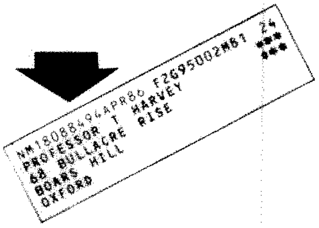

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Figure 1.

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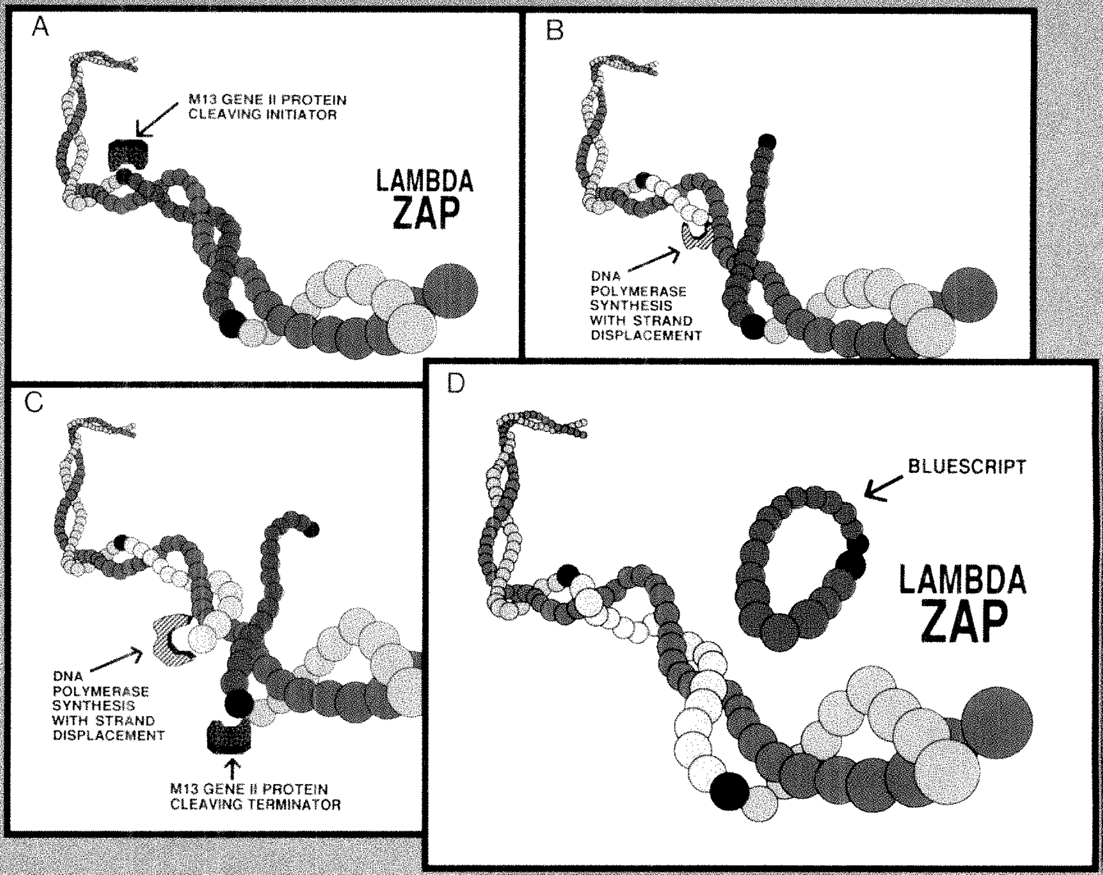

A
M13 GENE II PROTEIN CLEAVING INITIATOR
LAMBDA ZAP
B
DNA POLYMERASE SYNTHESIS WITH STRAND DISPLACEMENT
C
DNA POLYMERASE SYNTHESIS WITH STRAND DISPLACEMENT
M13 GENE II PROTEIN CLEAVING TERMINATOR
D
BLUESCRIPT
LAMBDA ZAP

A. M13 gene II protein recognizes the signal for the start of DNA synthesis and cleaves the DNA strand at the M13 recognition site.

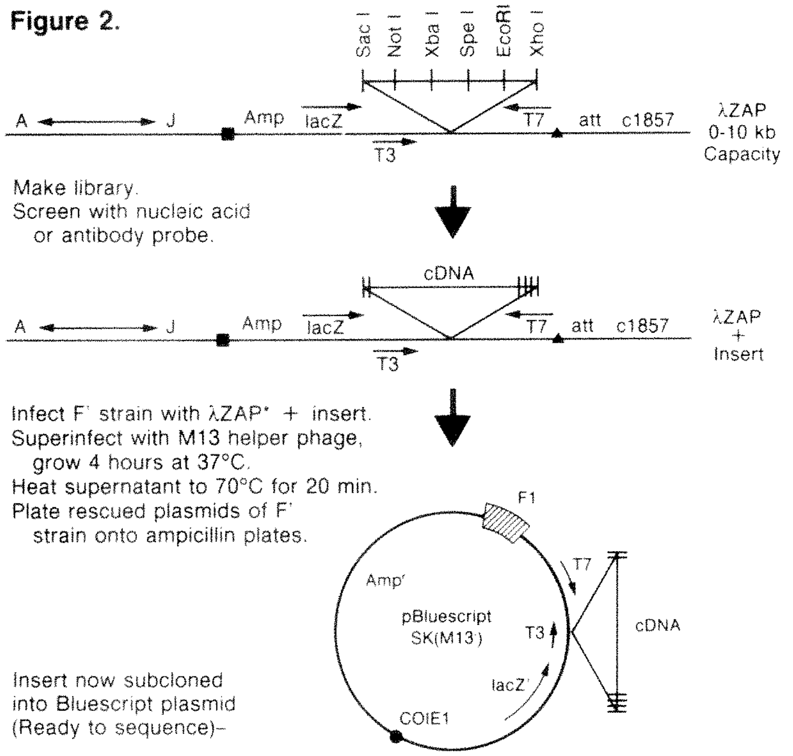
B. DNA polymerase synthesizes a new strand, displacing the existing strand.

C. M13 gene II protein recognizes the signal for termination of DNA synthesis and cleaves the DNA strand at the M13 recognition site.

D. M13 gene II protein converts the displaced strand of cDNA into a closed circular plasmid.

Breakthrough

Figure 2.



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KEY

A⟵J	: lambda structural genes
att	: lambda attachment site
■	: F1 initiator
▲	: F1 terminator
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(2) DNA and RNA sequencing
(3) Site-directed mutagenesis
(4) Synthesis of RNA probes
(5) Expression of proteins
(6) Isolation of single-stranded DNA
(7) Production of capped RNA transcripts
(8) Translation of capped transcripts.

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(1) Short, J.M., Fernandez, J.M., Huse, W.D. & Sorge, J. (1987) Manuscript in preparation.
(2) Fernandez, J.M., Short, J.M., Renshaw, M., Huse, W.D. & Sorge, J. (1987) GENE (submitted).

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2 pTGFβ2 is a structurally distinct second form of TGFβ (sequence homology 70% in the first 50 a.a. to hTGFβ/pTGFβ1). When assayed as above, it gives the same ED_{50}s on NRK and AKR cells. However, some cell lines can distinguish between pTGFβ2 versus pTGFβ1/hTGFβ.

3 Human platelet derived growth factor h(PDGF) is assayed on Balb/c-3T3 and NIH-3T3 cells for H^3-thymidine incorporation as per Raines and Ross, Meth. in Enz. *109*, p749.

4 Porcine PDGF is structurally very different from hPDGF (i.e. differs in M.W., subunit composition, N-terminus), and is biologically less active in thymidine incorporation assays.

5 Both the acidic and basic forms of bovine fibroblast growth factor are isolated from brain and assayed for H^3-thymidine incorporation on NR6-3T3 cells after Gospodarowicz, *et al* JBC, *253*, p3736, 1978.

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nature

How to get back from the dead

Two developments in the British academic scene offer a remote chance that British universities may yet be revived. But there is no time for leisured consideration of what to do.

The question whether the British university system will be able, like Lazarus, to rise from the dead will not be settled by the announcement last week that academic salaries are at last to be increased (see p. 567) or by the recommendations of the government committee under Lord Croham which, for the past year, has been brooding on the organization of the University Grants Committee (UGC), the conduit for the government's direct support of the universities. (The committee's report will have been published before this issue of *Nature*, but the essence of its proposals has been well flagged in advance.) More pay may persuade some able academics to postpone decisions either to emigrate or to look for more rewarding jobs in other sectors of the British economy. In the long run, there may be more hope in the proposal that UGC should be organized so that its chairman is an independent part-time person who is not an academic. But jaundiced bystanders will rightly suspend judgement about the long-term benefits of these developments, the second of which has not yet been accepted by the government.

British universities, and the system of which they are a part, have been put into a dream-like environment by the events of the past decade. It has been a curiously British process which began ten years ago, before the present government came to office, when the then government sought help from academics in containing the costs of student support and in planning for the demographic prospect of a declining population of young people (now a reality). It is understandable but not forgivable that, after a period of rapid publicly supported expansion, the university system should have hotly rejected the notion that budgets might be constrained. What those responsible appear not to have appreciated is that the government was then, as it still is, the paymaster.

Economies

Since the election in 1979, that proposition has been self-evident to all. The process of making the public cost of the university system shrink has nevertheless been accompanied by such a spate of uneconomic economies that there is no particular reason why those who work in the system should believe that the money that keeps their institutions alive is of the same currency as that the government uses to pay a weekly dole to more than three million people who are unemployed. Many universities and university departments have been prevented from taking as many students as they could usefully have taught. Some, considered to be too small when standing on their own, have been compelled by outside pressure to merge with others only to discover that student demand is stagnant. Many academics have been invited to leave early on generous terms, and have then been reemployed as part-time teachers. In the current academic year, most British universities are outspending their incomes in the fond, but unfounded, belief that some future government or UGC will bail them out. In a climate in which vacant posts are left unfilled for as long as possible, is it any wonder that younger academics prefer to work elsewhere, where opportunities for doing things for the first time persist?

British universities underestimate the damage being done to their well-being by the inclination of ambitious academics to move elsewhere. Part of the trouble is that the numbers are not absolutely large — perhaps only a few hundreds each year. Most often, these people pack their bags and go elsewhere only when their promise has been recognized by other institutions able to offer posts promising facilities for research, some security and much opportunity. The direct consequence is that some of the more promising research projects in British universities are transferred elsewhere, but the indirect effects may be even more damaging. Who can suppose that the spirits of those left behind remain as high as they should be? And who can be surprised that now, after a decade of attrition, British universities are hard-pressed to recruit able graduate students from their undergraduates and able postdoctoral people from among their PhDs? One way or another, the academic profession in Britain must head off the danger that it will become a scorned profession.

Prospects

Last week's salary settlement will help a little, but not permanently. British academic salaries will even now not compete with those to be had in comparable occupations. And while it remains the case, as politicians (in Britain, even more poorly paid) are never tired of pointing out, that academic freedom and even vacations are worth a great deal, academics are acutely conscious that these intangibles will not pay grocery bills. In any case, individuals' immediate benefit will be offset by the way in which their institutions will have to dig deeper into their red ink to pay the extra, while the prospect that the government will not for the time being contribute to the extra cost after the first three years can only make financial planning seem even more an exercise in the unreal. The only potential long-term benefit of the settlement is that the salaries that universities may pay to individual professors will not in future be constrained. That, potentially, is a means by which some universities might keep some able academics on their books. They will pay for that freedom by the requirement that the performance of less senior people should be more often, and more explicitly, assessed, which will be no bad thing.

But will universities be able to take advantage of this freedom offered by the new pay settlement? Three pre-conditions will have to be satisfied. First, universities will have to devise some means collectively of making wise decisions about the quality of outstanding colleagues; their general indecisiveness on issues of the quality of research in the past few years does not promise well. Second, UGC (whether or not remodelled) will have to be prepared to deal generously with the better universities, embarking on the pursuit of excellence without shame. Third, and even further ahead, all parties to these bargains will have to acknowledge that by helping universities make bargains with individual academics, they are assisting in a process whose logical conclusion is a degree of autonomy that British universities do not now enjoy.

This is where the remodelling of UGC is indispensable. Under the present system, UGC tends towards equitability between institutions. Although it has begun on the process of distinguishing between institutions on the basis of their reputations in research, its first essays in this direction have been modest and uncertain, while the Secretary of State for Education and Science has made it plain that he would not allow the process of discrimination to go so far as to threaten the existence of weaker institutions. The potential benefit of a UGC reorganized as a council on Croham lines is that it might be willing to take hard decisions of this kind, fighting the government for its right to be discriminatory between the good and the less good if that is what the circumstances require. The obvious danger is that such a council might shoot off in an eccentric direction, the pursuit of what is fashionably called 'relevance', not excellence, for example. On balance, the risks are worth running for the chance that greater autonomy will emerge.

Overlap

None of this will absolve the British government from worrying about higher education. For twenty years there has been an anomalous division of higher education into two sectors, one called the 'private' sector which is supported directly by central government through UGC and one called the 'public' sector because it is supported by central government through local authorities. Especially if universities are compelled by financial pressures to become more diverse in what they do (which would be welcome), and if Mr Kenneth Baker, the Secretary of State for Education and Science, succeeds in detaching the institutions called polytechnics (a large part of the public sector) from the local authorities, there will be even more overlap between the systems than there is at present. Will it then make sense to let them coexist as ostensibly different kinds of institutions? That is one issue to be settled soon. Another is whether it makes sense that UGC should continue to be the conduit for a notional £600 million a year intended for the support of academic research when the research councils, formally responsible for the same causes, are increasingly incapable of supporting projects which, by lapsing, help to drive the iron deeper into the heart of British academic science. Why should not the government settle these issues while deciding how to respond to Croham? That way, there might be a chance of returning the system to good health. ☐

Choose your timescale

US researchers, under pressure to be more productive, may find fewer benefits in plurality.

ALL governments believe that research, and particularly scientific research, is potentially a prolific source of wealth and, perhaps even more important, of international advantage. Even academic researchers know that. Most governments have also followed their declared precepts in arranging that research should be conducted in an orderly fashion. The most successful framework so far devised is that still in being in the United States, where the bulk of what is spent on what researchers do is spent by agencies which pretend they have a practical interest in the outcome. In reality, of course, the need for the US Department of Energy to know whether there is a *top* quark, a question that may be settled when its next or next-but-one accelerator is working, is no more compelling than the need that NASA should have a more rounded understanding of why the Universe expands. The framework succeeds only because the sponsors are tolerant of their pensioners' enthusiasms. In places such as Japan, where government agencies are required to be less flighty, public sponsorship of research is less catholic and also less productive.

For how long, in circumstances like these, can the casually creative system in the United States survive? There are both pluses and minuses. This week's report (see p. 564) that the US Navy cannot afford the $400 million-odd that would have been required to build a better sea-surface satellite monitor is a far cry from the days (in the 1950s) when the US Office of Naval Research was investing in molecular biology on the grounds that nobody could deny the possibility that aircraft — US Navy aircraft, of course — would one day be flown by appropriately coded nucleic acid molecules. Studies of the behaviour of porpoises were at the same time encouraged on equally free-thinking grounds. It is true that liberality of this kind has always gone hand-in-hand with even more generously supported projects of a strictly practical character, but inter-agency competitiveness has been a constant spur to farsightedness. That is another reason why the US Department of Energy's willingness to take the next big particle accelerator under its wing is to be welcomed; the project may do little for the state of the domestic oil-producing states, but it will ensure that some other agency (the National Science Foundation, perhaps?) does not get hold of it. Researchers know well enough the benefits of this lavish plurality.

That is why the events of the past few weeks in the United States are mildly disturbing. The new budget, at the beginning of the year, is above all tidy. The National Science Foundation (NSF) is destined to grow. Other agencies, such as the National Aeronautics and Space Administration (NASA), will get more than last year if only Congress agrees, but will have more of the money tied up in specific tasks (building another shuttle spacecraft, for example). It could easily be that the pluses, especially the more open recognition that NSF should prosper so as to support more and better research, will be offest by the minuses arising from the workaday mould into which the mission-oriented agencies are being forced by the budget problems of the United States. It is far too soon to begin worrying that the condition of research in the United States will soon resemble that in, say, Britain, for the margins for free spending by US agencies are still very broad. But there are unwelcome signals in the single-mindedness of the mission-oriented agencies that could yet force US researchers to follow the short-term conventions common elsewhere. Ironically, if the worst should happen, the damage will have been done in the name of good government. ☐

What does ABM mean?

The prospect of a transatlantic quarrel over the Anti-Ballistic Missile Treaty cannot be avoided.

IT appears that the US administration is drifting towards serious disagreement with Western Europe over the deployment of some elements of the Strategic Defense Initiative (SDI). The difficulty is the wish of many in and just outside the administration to have something of SDI in place before the end of President Reagan's term in office, roughly 22 months from now (see *Nature* **325**, 470; 1987). The wish is easily understood, even by those who do not share it. The puzzle (to Western Europeans) is that the United States seems not to understand, let alone to have anticipated, why the issue is so important that even the secretary-general of the North Atlantic Treaty Organization, Lord Carrington, should have written to Washington for an explanation of what is going on.

The origins of the Anti-Ballistic Missile (ABM) Treaty of 1972 refer, then, at the beginning of what turned out to be 70 per cent of a decade of detente, the United States accepted that the Soviet Union had the capacity to defend a few targets (Moscow in particular) against some missiles, that the cost and difficulty of its own development of a high-acceleration missile called SPRINT would not be worth the candle and that there was strength in the general opinion that, to the extent that ABM defences must undermine the now-traditional theories of deterrence by the threat of mutually assured destruction, they are best avoided. President Reagan made a good debating point in March 1983 when he argued that it must be preferable to prepare to defend one's people against attack than to prepare to attack potential attackers. But the issue has never been debated. That is why, late in the day, there must now be an argument. ☐

Europe alarmed at US plan for space developments

● SDI could break ABM treaty
## ● Space station — a military base?

Washington & London

European governments are increasingly concerned by what they see as the drive by the US military to dominate space through the Strategic Defense Initiative (SDI) as well as by a renewed interest in the space station programme.

Mr George Schulz, the US Secretary of State, has met European delegates during the past week in an attempt to allay their fears over what appears to be a new political momentum behind an early deployment of SDI. And representatives of the European Space Agency (ESA) have been in Washington trying to determine the motives behind the plans being developed by the Department of Defense (DoD) for the space station.

The Belgian foreign minister and present head of the Council of Ministers of the European Communities, Mr Leo Tindemans, claimed that there is extensive unease among European governments about SDI. The Anti-Ballistic Missile (ABM) Treaty, now 15 years old, restricts full deployment, the European governments claim, and they want research on the SDI project to be curtailed to comply. The United States appears not to agree.

But the Europeans are more than surprised at the revived interest in the space station, previously considered an exclusively civil project. The SDI ambitions of the United States and the new plans for the station, many European observers fear, could exert pressure on the North Atlantic Treaty Organization and scupper any hopes of early agreement with the Soviet Union on restricting the use of nuclear weapons. The Soviet Union has consistently tried to arrest the progress of SDI and considers the project to be substantially less advanced than is claimed by the United States.

European participation in the US project to build a space station will depend on a meeting arranged for 11 and 12 February in Washington with all international participants. An ESA delegation will examine several issues raised in a document prepared by NASA after discussion with US government agencies on the future military interests in the space station.

Negotiations planned for last month were delayed at the request of DoD to give it time to review its interest in participating in the multinational project.

The new US bargaining position was worked out late on the evening of 4 February, and transmitted to the countries involved. Although no details of the new US position were released, NASA administrator James Fletcher told a House of Representatives committee on Thursday that the space station would still be reserved for peaceful purposes.

In other testimony, Fletcher maintained that the administration's request for $767 million for 1988 for the space station would meet the goal of operational capability "in the mid-1990s". But it is certain that the current $8,000 million estimated cost for the space station will go up. NASA has completed two separate analyses of space station costs following the completion of the preliminary design and definition phase of the project. The details are being worked out with the White House Office of Management and Budget, but the cost is likely to rise as high as $13,000 million, if not higher.

A spokesman for ESA said last week that the purpose of this week's meeting is less to negotiate the US draft than to study the practical consequences of what NASA now proposes should be the role of the US Department of Defense in the design, management and operation of the space station. All the member governments will be represented on the ESA delegation by at least one member. The political implications of the draft document will be decided only when the delegation has returned from the United States. It will then be open to ESA governments to seek changes in the draft plan.

ESA hopes that it will still be possible to agree by spring on the development of its Columbus project as an adjunct to the space station proper. But the timetable has been thrown awry by DoD's unexpected declaration of interest, last December. ESA hopes for an agreement among its members in April, in time for some industrial contracts to be let by midsummer.

Although the Columbus project, a manoeuvrable vehicle based on the space station, may appear to be a separate project, it will require a dedicated module integral with the space station as a whole.

European diffidence about DoD's possible involvement in the project centres on the extent to which scientific observations by Columbus and its crews may be restricted by US military requirements and on the unwillingness of some European governments to be seen to be contributing to a US-inspired military project in space.

Joseph Palca & Bill Johnstone

Embarrassing failure for Soviet launches

London

During the past few weeks, the Soviet Union has had problems with two of its Kosmos satellites. According to the Kettering monitoring group, K-1813, launched on 13 January, was destroyed after a failure of the rocket motor, and K-1817, launched on 30 January, failed to boost into a stable orbit and re-entered prematurely. In spite of the current policy of *glasnost* (openness), the Soviet space planners have been very reticent about the two Kosmos failures.

They have reason for reticence. Since the Challenger disaster last year, the Soviet Union has offered the services of its Proton launcher to countries wishing to launch communication, meteorological or experimental satellites on a commercial basis. The proposal has the special backing both of Mr Mikhail Gorbachev, who sees it as a first step to his plans for the 'star peace' based on international research projects which he expounded in India last year, and by Prime Minister Nikolai Ryzhkov, who in an interview with the TASS agency last month stressed the "versatile and reliable" nature of the Proton. In fact, up to the end of last year, according to official Soviet data, there had been seven failures of Proton out of 102 launches, and (in the absence of an official statement) it is impossible to pinpoint the cause of the two recent Kosmos failures.

Soviet astronauts — still smiling

Even without the commercial launches, however, Soviet cooperation programmes are booming. French and Syrian cosmonauts have been in training for some time, and the Bulgarians have at last been granted a re-run (scheduled in 1988) of their April 1979 mission, which was aborted when the Soyuz transporter failed to link up with Salyut station. Unmanned missions with international cooperation include a study of the surface of Phobos, X-ray astronomy, and research into solar activity. One difficulty for western scientists wishing to supply state-of-the-art equipment to joint missions (which could possibly run foul of the CoCom embargo on the export of sensitive technology to the Socialist Bloc) seems to have been partially overcome. In the case of commercial launches, the Soviet side has unequivocally promised that sealed experimental packages would remain sealed, and that there would be no attempt to "pump out technological secrets" from them. The same assurances could surely be extended to joint experimental missions.

Vera Rich

Wellcome gears up production of AIDS drug

London

CONFIDENT that the first approval for the use of its anti-AIDS drug, Retrovir (trade name for AZT), is just around the corner, Wellcome plc is busily preparing itself for the anticipated demand. At a capital cost of £17 million, additional general purpose plants are being constructed both at Dartford, Kent, and at Greenville, Tennessee.

But even with their new production capacity, the company may not be able to meet estimated worldwide demand, in which case, presumably to avoid the kind of criticism that followed its initial allocation of the drug solely to the United States, Wellcome would allocate supplies on a geographical basis according to the number of reported cases.

Wellcome makes the drug (3'-azido-3'-deoxythymidine; AZT), by chemical synthesis from thymidine, which in turn is chemically synthesized by another company, whose identity is secret. Both companies are scaling up their chemical syntheses, which involve sixteen steps.

By May this year, Wellcome expects to have enough of the drug to meet the demand for its use in AIDS (acquired immune deficiency syndrome) patients who are seriously ill with pneumonia caused by *Pneumocystis carinii*, a group that has responded well to the drug in clinical trials. But if worldwide approval for use of the drug in patients with serious AIDS-related complex (ARC) was also soon granted, supplies would not meet demand. On existing figures there are about 15–20,000 patients in each category, needing an average of about one gram a day of the drug continually.

Approval for use of the drug in one or both conditions is expected from the US Food and Drug Administration in "five or six weeks", according to a Wellcome spokesman. And the UK Committee on Safety and Drugs is considering an application based on the same data. Wellcome will be submitting similar applications to other major European countries soon. In addition, clinical trials will test the value of the drug, alone or in combination therapy, in less severe cases of AIDS or ARC.

Responsibility for Retrovir production changed hands last week with the resignation of Dr Ronald Cresswell, who became the director responsible for coordinating Wellcome's US and UK research and development after the departure of Sir John Vane. No direct replacement has been made. Instead, Dr Trevor Jones takes over UK responsibilities and Dr Howard Schaeffer runs the US side.

Peter Newmark

US Navy torpedoes plan with NASA for ocean sensing satellite

Washington

THE US Navy is to scrap NROSS, the Navy Remote Ocean Sensing Satellite. The decision has not only dealt a telling blow to the future of the Navy's global weather and oceanographic forecasting, but has caused consternation among the Navy's partner agencies. The decision has forced NASA (National Aeronautics and Space Administration) to look for a new vehicle to carry a scatterometer, considered essential to the future of civilian oceanographic research.

NROSS began life in the 1985 fiscal year when it received formal budgetary approval. The satellite was to travel in a Sun-synchronous polar orbit, carrying a low-frequency microwave radiometer to provide all-weather high resolution measurements of sea surface temperature as well as an altimeter to measure waves and eddies. The Navy planned to include its own scatterometer on NROSS, but NASA offered to provide the Navy with NSCAT, an improved version of the scatterometer flown on the successful 1987 Seasat mission.

The Navy accepted and signed an agreement with NASA in July 1985. NSCAT transmits 14-GHz pulses towards the Earth, recording the reflected microwave signals. Ground-based computers interpret satellite telemetry to construct a map of ocean winds. NSCAT was to provide scientific data for NASA and operational data for the Navy.

The Navy's plans have been scuppered by rising costs in 1986. By July last year, the estimated cost of the satellite was $420 million, $150 million more than expected. By November, Assistant Secretary of the Navy Melvyn Paisley had called for a study of ways out of the dilemma, and the NROSS programme office presented three options at a meeting on 11 December: find the money from somewhere else in the Navy's budget, replace the low-frequency microwave radiometer with one costing less or cancel the programme.

Because the alternative radiometer would not work under cloudy conditions and with the prospects of finding the extra money only slim, it was decided to cancel the programme.

The oceanography "union" in the US Navy does not have the clout of others, such as those advocating construction of aircraft carriers or destroyers. There are also indications that the large cost increase for NROSS should not have come as a surprise, as the Navy had modified its requirements for the satellite. Thus, responding to concern created by the shuttle accident, the Navy had attempted to make contractors liable for mission failures, to which contractors had responded by raising costs.

Attempts by supporters of NROSS to intercede have so far failed. Both NASA administrator James Fletcher and National Science Foundation director Erich Bloch wrote to Navy Secretary John Lehman urging him to retain the programme. Carl Wunsch, James O'Brien and Walter Munk, three of the four holders of Secretary of the Navy Research Professorships, also protested. Unpersuaded, Lehman agreed on 15 December to scuttle the satellite.

Following the Navy decision, Fletcher wrote to William Taft IV, deputy secretary of defence on 17 December, saying that "the cancellation of NROSS would set a bad precedent for other ongoing and future cooperative programs between DOD [Department of Defense] and NASA".

In a conciliatory response dated 20 January, Taft declined to change the decision but suggested that NSCAT might find a home in the Defense Meteorological Satellite Program, an option already being explored by NASA. The Air Force has now begun a study of that proposal, as well as the possibility of including NSCAT in DOD's Space Test Program. These options could be acceptable to NASA, since they would permit NSCAT to fly concurrently with TOPEX/POSEIDON, the joint NASA–French oceanography satellite due to be launched in December 1991. NASA is also investigating putting NSCAT on TOPEX.

Cancelling NSCAT, estimated to cost $130 million, would be expensive for NASA. The agency has already spent $30 million on the project, and an additional $30 million would be needed to end the programme in an orderly fashion. But William Townsend of NASA's Oceanic Processes Program says he is "extremely optimistic" that something can be worked out to allow NSCAT to complete its mission.

Wunsch calls the Navy decision on NROSS "extremely short-sighted". He argues that cancelling NROSS will have severe repercussions for the Navy in the next 10–15 years when forecasting global oceanographic conditions will be critically important for new weapon systems.

D. James Baker, president of the Joint Oceanographic Institutions, says wind data provided by NSCAT are essential for WOCE, the World Ocean Circulation Experiment. Baker says a delegation of oceanographers is hoping to meet Navy officials soon to discuss the future of Navy's oceanographic programme.

Joseph Palca

Canada demands action from United States on acid rain

Washington

THE Canadian government's quarrel with Washington over acid rain has moved into a new and more acrimonious phase, with the Canadians demanding immediate action. At a meeting on 21 January, the Canadian Prime Minister, Brian Mulrony, gave US vice-president George Bush what is described as an "earful" and declared that the issue of acid rain will be "at the top of the agenda" for his meeting with President Ronald Reagan in April.

US efforts so far to reduce acid rain were criticized in a report issued last month by Canada's environment ministry. The US State Department has responded with another high-level review while efforts to improve control continue to focus on the long-range development of new and affordable technologies to reduce the pollution from coal smoke, with no immediate reduction of the amount of sulphur dioxide crossing the border.

US policy on acid rain continues to ignore the Canadian demand that it should control the pollutants responsible for acid rain, despite the report in January 1986, by two specially appointed envoys, which persuaded the Reagan administration to agree in principle that acid rain was a serious problem. Since then, Canada has been waiting for policy changes in the United States, but the new budget contains no new programmes to reduce sulphur dioxide emissions from coal-fired plants.

Instead, the US administration points to its recent clean-coal technology legislation, which is intended to reduce the cost of new techniques for removing pollutants from coal smoke. But no immediate reductions are required, while the new technology will probably be applied only as older power plants need refurbishment.

The Canadians are not alone in calling for changes in US policy. Two bills introduced in the Senate this month would require a significant tightening of US air pollution controls. The bills were backed by testimony from physicians such as Dr Richard Narkewicz, president-elect of the American Academy of Pediatrics, who stated that "the ingredients of acid rain adversely affect the respiratory tract and consequently the quality of health for children" and "contribute greatly to mortality rates". Further testimony indicated that air pollutants such as sulphur dioxide, nitrogen oxides and ozone put many others at risk, including people over 65 and sufferers from asthma and heart disease.

New political alignments in the Senate may prove to be insurmountable obstacles to the proposed legislation. Senate leaders from coal-producing states, for example, will probably provide strong opposition.

Although the administration no longer disputes the causal connection between sulphur dioxide pollution and environmental damage, it asserts that it is unclear at what rate the damage is being caused. "The argument can be made", said Chris Rice of the Environmental Protection Agency (EPA), "that the damage we see now is due to sulphur dioxide emitted in the 1950s or 1960s". But Debbie Sheiman of the Natural Resources Defense Council says "the administration seems to be using delaying tactics" to avoid using pollution control technology now available.

Renewed Canadian demands for US reductions of acid rain have directed attention to Canada's own acid rain reduction programme. The seven provinces east of Saskatchewan agreed in 1985 to reduce sulphur dioxide emissions by 50 per cent by 1994. Stricter emission controls on automobiles will take effect in September this year.

But some in the US Congress believe the Canadians are being hypocritical, as they have yet to begin installing pollution control devices on their own smokestacks. A Canadian government spokesman rejects this charge as irrelevant, because most sulphur dioxide pollution in Canada results from non-ferrous smelters, not power plants. The most effective (and most often used) control device for such a smelter is to shut it down, as was done with the International Nickel Company's plant at Sudbury, Ontario, for example.

The question of what will happen to sulphur dioxide emissions in Canada when economic conditions favour the continued operation of these plants remains open.

Steven Dickman

Atomic power struggle under way in India

New Delhi

THE appointment of a successor to Dr Raja Ramanna, retiring chairman of the Indian Atomic Energy Commission (AEC), has been postponed at the last minute in the wake of threats of resignation and an internal power struggle between top scientists at the Department of Atomic Energy (DAE). Whether an engineer or a physicist should be made head of the commission is the subject of a controversy that has brought to the fore the factional politics within DAE and the schism between research scientists and engineers. With two contenders vying for the post, an embarrassed Prime Minister, Mr Rajiv Gandhi, who is also in charge of atomic energy, has asked Ramanna to continue as chairman for a further month.

Mr Rajiv Gandhi

One of the aspirants for the post is Dr P.K. Iyengar, the director of the Bhabha Atomic Research Centre (BARC), which is the principal agency responsible for research and development. Iyengar, a reactor physicist and a key figure in the design and testing of India's first nuclear device, was Ramanna's student and his favourite for the post. The other contender is Dr M.R. Srinivasan, an engineer, who is in charge of DAE's nuclear power board, which is responsible for the design, construction and operation of nuclear power plants. Both Iyengar and Srinivasan are members of AEC.

Ramanna, who was to have retired on 31 January, was not told of his successor until the previous evening when he was told to hand over to Srinivasan. Twelve hours later the order was withdrawn, apparently because Iyengar threatened to resign. Ramanna was then asked to continue as chairman until the end of February and the prime minister presumably hopes that the delay will give him time to pacify Iyengar. Scientists at DAE, upset over the last-minute change, have openly accused Ramanna of blackmailing the government and stalling the appointment of Srinivasan, whose name had been proposed by a special selection committee.

Gandhi's handling of the issue has been attacked in the newspapers, which claim that it has lowered his credibility within the scientific community. Similar criticisms were voiced a few months ago when Gandhi abruptly removed Dr S. Varadarajan from his post as director-general of the Council of Scientific and Industrial Research.

The bitter taste left behind by the struggle over the succession at AEC will take time to wipe out, but Gandhi has already taken steps to bring peace among the warring scientists. He has held separate meetings with Iyengar, Srinivasan and Ramanna, and the name of the new AEC chairman is expected to be announced soon.

K.S. Jayaraman

Ten colleges combine forces to strengthen Earth Sciences

Washington

TEN small liberal arts colleges which have already had a disproportionate impact on graduate research in the Earth sciences are hoping to improve that record with a $450,000 grant from the W.M. Keck Foundation. The grant to the ten-college consortium will permit joint faculty–student research projects as well as short-term faculty exchanges.

Four-year liberal arts colleges have regularly provided a large number of graduate students to US universities (see *Nature* **322,** 203; 1986). This is borne out by a 1982 survey of 876 undergraduate institutions conducted by Franklin and Marshall College, a member of the ten-college consortium. The survey showed that although the ten colleges now banding together produced less than one per cent of the total number of first degrees in the institutions surveyed, they accounted for more than a quarter of the total graduate degrees in the Earth sciences.

The colleges in the consortium are Amherst (Massachusetts), Beloit (Wisconsin), Carleton (Minnesota), Colorado College, Franklin and Marshall (Pennsylvania), Pomona (California), Smith (Massachusetts), Whitman (Washington), Williams (Massachusetts) and College of Wooster (Ohio).

Reinhard Wobus, a geology professor at Williams College in Williamstown, says the strength of the small colleges comes from the opportunities for close faculty–student interactions. The Keck grant will offer top undergraduates in the Earth sciences a chance to participate in professional research. In the spring following each summer's research projects, there will be a conference to discuss the findings.

Wobus says a similar programme sponsored by the National Science Foundation in the early 1970s involving Williams and three neighbouring colleges proved the value of such experience. If there is a problem with the new programme, Wobus believes it will be logistical. The ten colleges are scattered throughout the United States.

Improving instructional skills is also a goal of the consortium. Faculty from the colleges will go on three-day visits to the other institutions to observe classes and conduct guest lectures.

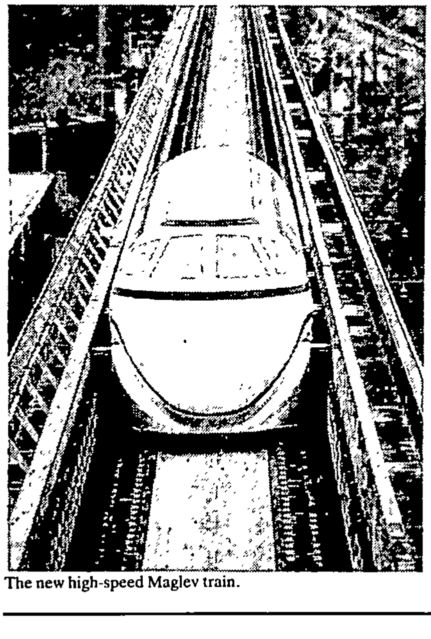
The first research projects will begin this summer. One, directed by Wobus, will collect and analyse material thrown up by volcanoes in the Thirtynine Mile volcanic field in central Colorado. Other projects this summer will be a study of ancient stream systems in Montana and carbonate sedimentation on San Salvador island in the Bahamas. Although the programme will be limited to three research projects this summer, the number should double in 1988.

Joseph Palca

Japan sets world record for levitation

Tokyo

JAPANESE National Railways (JNR) established a world record last week when the magnetically levitated (Maglev) train reached a speed of 400 km an hour with a crew of three abroad. The successful experiment sets the stage for test runs of a prototype commercial vehicle later this year.



The new high-speed Maglev train.

The new record was made possible by doubling the electrical capacity of JNR's 7-km test track at Miyazaki, Kyushu, to 6,000 V, and shattered the previous record of 350 km per hour for a manned train set by West Germany's Transrapid 06 vehicle in December 1985.

Japan's MLU-001 experimental train 'floats' on on-board superconducting magnets and is propelled forward by magnetic coils set in the sides of the trackway. A prototype commercial vehicle capable of carrying 44 passengers at a speed of up to 420 km an hour will begin tests in March on the Miyazaki test track, and Yoshihiro Kyotani, manger of the Maglev project, hopes that a new 50-km trackway for the prototype will be constructed by the end of this decade. Seven prefectures, including Hokkaido, Yamanashi and Saitama, have already expressed interest in the new line which will cost at least Y150,000 million ($1,000 million) to build and can be put into commercial use once experiments are completed.

David Swinbanks

Japan under fire from US on supercomputers

Washington

SUPERCOMPUTERS are the latest Japanese products to come under scrutiny by the Office of the US Trade Representative, this time at the behest of the White House. The results of an investigation begun last year will be disclosed next month.

The President's Trade Strike Force, an informal body at cabinet level, asked for the Trade Representative's investigation, apparently in response to the first purchase of a Japanese supercomputer by a US corporation. The Houston Area Research Center (HARC) — a non-profit corporation designed to link government and industry with the research capabilities of local universities — announced last year that it was acquiring a Nippon Electronics Corporation (NEC) SX2 supercomputer for $20 million. The prospect of Japan successfully invading another market hitherto dominated by indigenous companies evidently provoked the request for an investigation.

The investigation is being carried out in parallel with the Market Oriented Sector Selective (MOSS) talks with Japan, which deal with the Japanese markets from which the United States has been excluded. These include pharmaceuticals, electronics, lumber, automobile parts and telecommunications equipment. Supercomputers have now been added to the electronics segment of the talks which apparently are going 'slowly'.

Two US companies now manufacture supercomputers; Cray Research Inc. and Control Data Corporation (CDC). Cray has sold six supercomputers in Japan, the latest of them last month, when Honda bought a $5.5 million Cray X-MP/1. The X-MP/1 has only a quarter of the power of its big cousin, the X-MP/4, but is still 50 per cent more powerful than the Cray 1. So far, CDC has not sold any supercomputers in Japan.

Bob Gaertner, vice president for communications for Cray Research, says Cray's problems in Japan have been chiefly in sales to government agencies and universities. Cray's Japanese sales total $60 million, all to the private sector. Gaertner says Cray wants to expand Japanese sales and is "anxious to have a more open market". So far, Cray has sold 24 of its top-of-the-line supercomputers worldwide; 18 X-MP/4s and six Cray 2s.

HARC's SX2 can perform 1,300 million floating point operations per second (1.3 gigaflops). The new CDC ETA/10 operates at only 10 gigaflops, and costs between $15 and $21 million depending on how it is supplied.

Joseph Palca

Britain's doctors in legal wrangle over confidentiality of records

London

BRITAIN's medical profession is embroiled in the debate over the ethics and legality of withholding information from patients. Tests to determine the health of fetuses and others to identify AIDS (acquired immune deficiency syndrome) carriers has highlighted deficiences in British medical law.

The fetal test debate was originally provoked by a report from India of concern over the "steady increase in the incidence of female feticide", brought about by abortions inspired by information derived from amniocentesis testing (*Nature* **324**, 202; 1986). The controversy continued with correspondence, in response to the Indian report (*Nature* **325**, 190; 1987), from British cytogeneticists who have been withholding sex information culled from amniocentesis.

The debate has been reopened by the council of the Association of Clinical Cytogeneticists, which has become increasingly concerned over the potential misuse of amniocentesis data. Some members of the association have advocated withholding fetal sex details from parents who may demand abortion if the fetus is of the 'wrong' sex.

Last week, the council sought to determine the scale of the problem. Such evidence as could be acquired was 'anecdotal' and the association has been unable to issue definite guidelines. The full legal implications of withholding information from patients are to be investigated; in the meantime, the guidelines will be those agreed between "each regional laboratory and regional clinical colleagues".

While the UK Abortion Act theoretically does not allow the abortion of a fetus on the grounds of unwanted sex, health authorities have become increasingly concerned that the problems of India may be repeated in Britain, particularly among certain ethnic groups. Because of the scale of the problem in India — in a recent study of 8,000 abortions, 7,997 fetuses were female — new legislation is being drafted to restrict the practice of amniocentesis and other methods of determining fetal sex.

The screening of all hospital patients for the AIDS virus has raised similar legal and ethical questions. The UK government is considering authorizing the 'blind testing' of all patients to identify the AIDS virus. No individual may be identified in the tests and the information will be used only to establish the spread of infection. The disclosure was made last week by the government's chief medical officer, Sir Donald Acheson, in evidence to the House of Commons Social Services Select Committee.

He said: "It is being considered by ministers as a possibility. It would help us very much in planning and getting an idea of the prevalence and trends of the infection."

The plan has provoked much controversy. The British Medical Association supports the plan but the Medical Research Council, which this week will give evidence to the Commons committee, has raised doubts about the legality of such a strategy. **Bill Johnstone**

- Britain has increased its annual budget for treating AIDS from £3 million to £7 million; the major regional health authorities treating AIDS believe a figure of £16 million would be a more realistic estimate of what is needed. □

Peace attempt with university pay deal

London

BRITAIN's university lecturers have been offered a two-year pay deal that will raise the average salary immediately by 16 per cent, backdated to last December, and by a further 7 per cent in March 1988. The figures are based on existing salary rates.

The executive is to meet this week to endorse or reject the offer although the negotiators have recommended its acceptance. About 47,000 academic staff and certain support staffs will be affected by the deal. The Association of University Teachers which had claimed 6 per cent, to be effective from last April, and 18 per cent from April this year, considers it the best deal under the circumstances.

According to Ms Diana Warwick, General Secretary of the AUT: "this offer is no remedy to recruitment and retention problems within our universities; it won't staunch the brain drain. There's been no pay increase in universities since 1985. It's clear that we have wrung out of the vice-chancellors the best deal we could get, within the miserly terms allowed for by the Secretary of State."

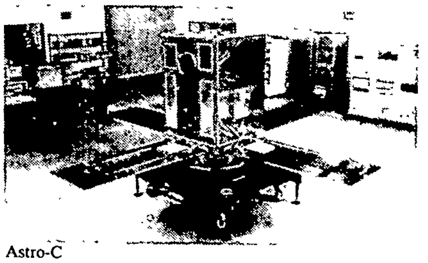
The monies will come from the £71 million of government funds allocated to the universities in a three-year plan — £40 million in 1987–88, £16 million in 1988–89 and the remainder in 1989–90.

The AUT will open negotiations on a new claim in April 1988, just one month after the award of the second tranche. The union insist that the deal is a two-year agreement and must not be construed as running after March 1988. Bill Johnstone

Japanese X-ray satellite goes up successfully

Tokyo

JAPAN's X-ray astronomy satellite, Astro-C, was successfully launched from the Kagoshima Space Center of the Institute of Space and Astronautical Science (ISAS) on 5 February. Developed jointly by Japanese, British and US scientists, the probe will monitor faint X-ray sources in the far reaches of the Universe.



Astro-C

The satellite, named Cinga (galaxy) in orbit, was carried aloft by a 28-m M-3SII solid-fuel rocket at 3.30 p.m. About 6 minutes later, the 420-kg satellite entered low Earth orbit, and radio transmissions from the probe were picked up after it had completed its first loop around the Earth at an altitude of 500–600 km.

Ginga, ISAS's third X-ray satellite, is the world's only operational X-ray probe following the demise of the European Space Agency's EXOSAT early last year. It carries eight identical large-area proportional counters, developed with groups at the University of Leicester and the Rutherford Appleton Laboratory in the United Kingdom, an all-sky X-ray monitor, and a gamma-ray burst detector, made in collaboration with a group at Los Alamos National Laboratory in the United States.

Collaboration between ISAS and the Leicester group stretches back seven years. Scientists have been exchanged under UK/Japan schemes for visiting scholars; the British side developed the hardware, Japan the electronics. Similar arrangements were made with the United States. The total cost of Ginga, including the MU-3SII rocket, is estimated at Y7,300 million (about $49 million).

The highly sensitive, large-area detectors (4,500 cm^2 in total) will be trained on distant X-ray sources, in particular quasars. The time-scale of X-ray fluctuations will give an indication of the size of the quasar nucleus and, according to Minoru Oda, director of ISAS, strong fluctuations would provide good evidence for the existence of a black hole in the centre of quasars that sucks in stars and interstellar material, releasing vast amounts of energy in the form of X-rays.

David Swinbanks

International spaceports planned for northern Australia

Sydney

Australia is to be the site for an international spaceport, if the governments in the top end of the continent have their way. A A$93,000 study commissioned by the Queensland state government is to recommend that at least 2,000 km^2 be set aside for a spaceport on the Cape York Peninsula, Australia's northernmost tip.

The size of the area is to allow plenty of space for horizontal take-off and landing rocketships, such as the British HOTOL or West German Sanger projects. Cape York is a vast and virtually unpopulated area near to the equator, at a latitude of 12°S and is close enough for rockets launched there to take full advantage of the boost provided by the Earth's spin.

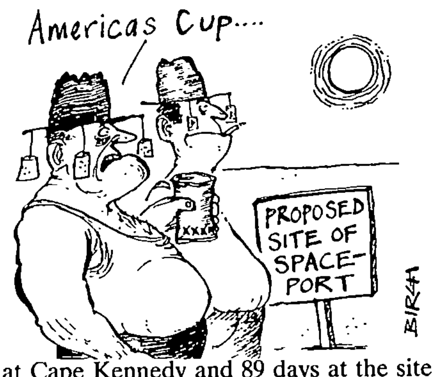
The same applies to the north coast of the Northern Territory. Having been prompted to consider the benefits of a spaceport, the Northern Territory government has dusted off a 1965 report by the European Launcher Development Organization (ELDO) which recommended a launch site near Darwin. A committee is reviewing the conclusions of the ELDO study to see if they are still valid today.

Both governments hope to attract launch operators from around the world. They consider that US rocket manufacturers will be moving into the launch business now that NASA is no longer launching commercial satellites.

The west Pacific location should be particularly attractive to Japan whose only launch sites are 30 degrees off the equator and closed for 10 months a year to placate local fishermen. They are also looking hopefully towards China whose only launch site at present is 40 degrees from the equator. The Queensland study is being conducted for Australia's Institution of Engineers by representatives of Hawker De Havilland, British Aerospace Australia, the University of Queensland, AUSSAT (the company operating Australia's domestic communication satellites), and the Commonwealth Scientific and Industrial Organization.

According to Mr Stan Schaetzel, leader of the study group and technical director at Hawker De Havilland, the report will recommend a site on the western side of Cape York facing the Gulf of Carpentaria. One great advantage of this site is its access to a variety of orbits without needing to overfly any centres of population. A standard due east launch would carry the rocket over the uninhabited forests of Cape York then out over the Pacific. For a polar orbit the rocket would head SSW, flying down over the Gulf of Carpentaria, across the continent and out over the Southern Ocean on a heading for the South Pole. Spent first-stage boosters would crash into the Simpson Desert somewhere to the east of Alice Springs. A course across Cape York and closer to the Queensland coast gives the spaceport access to a 28·5° orbit which corresponds to a due-east launch from Cape Kennedy and is the orbit NASA's space station will occupy. The US spaceports have very poor access to some orbits. Polar orbits cannot be reached from Cape Kennedy without wasting a lot of energy, whereas the other US launch facility at Vandenberg Airforce Base in California can launch into orbits but not due-east. Schaetzel points out that the US space agenda announced in 1986 calls for the establishment of a Moon base by 2010. The 28·5° orbit may well grow in importance in terms of the space station and space-based manufacture and for missions to the Moon and beyond.

Cape York's climate is favourable; only 39 launch days would be lost to electrical storms each year compared to 57 days lost at Cape Kennedy and 89 days at the site run by the European Space Agency (ESA) at Kourou in French Guyana, at present the only equatorial launch site.


I'd still rather have the Americas Cup....
PROPOSED SITE OF SPACE-PORT
BIRCH

Provision of infrastructure in such a remote part of Austalia is simplified by the fact that the prospective spaceport is right alongside what are perhaps the world's largest bauxite deposits. A nearby port capable of handling ships of up to 75,000 tonnes was built by Comalco Aluminium to support the workers at its mine there. Australia can also offer good radar cover and air traffic control as well as guaranteed political stability.

The world can be thought of as being divided into a land hemisphere and a water hemisphere. At present, all the world's launch sites are in the land hemisphere. Australia is in the water hemisphere. According to Schaetzel, at very least Australia an expect to play host to a spaceport which provides the pilots of future generations of shuttles with an emergency landing site.

Charles Morgan

Far Eastern Science Centre performs badly

London

The Far Eastern Science Centre of the Soviet Academy of Sciences, founded in 1970 as a focus for scientific and technical progress on the Soviet Pacific Coast is not living up to expectations, according to *Pravda*. Academician Skryabin, the academic secretary of the Soviet Academy of Sciences, blames the "sickness" of the centre on the lack of adequate "cadres" but locals (and *Pravda*'s Vladivostok correspondent, N. Bratchikov) say the distance from the academy's headquarters in Moscow makes proper control difficult.

New institutes of the Far Eastern Science Centre are built extremely slowly, and when completed, frequently stand unoccupied for lack of equipment. Only about 30–50 per cent of resources allocated are actually applied to work for the centre. The situation, bad in Vladivostok, is even worse in the outlying establishments of the centre. Laboratories, libraries and a computer centre specified in the plans have not been built. Furthermore, although some 9,500 staff (including 2,400 scientists) are now employed the number should be twice that. But the centre cannot attract qualified and motivated personnel until proper services are provided. At present 2,500 employees of the Far Eastern Science Centre are waiting for apartments and there are no hospitals, kindergartens or leisure facilities. The institutes do not solve such problems, nor do they provide proper working conditions. Laboratory space is only two-thirds of that given to a scientist at the Siberian Branch of the Academy in Novosibirsk, and allocations for instruments and equipment are only about half those at Novosibirsk.

In the mid-1970s, the centre was cited as an example of slackness in outlying scientific establishments. Mr Mikhail Gorbachev's recent visit to the Far East, where he repeated his calls for "restructuring", and "openness" has focused attention once again on the defects of the centre. However, the centre can claim a sound research record in many fields, especially Earth sciences, oceanology and ichthyology; its Kamchatka vulcanology centre is a world leader.

But the scientists say that if they are to do a proper job the centre should be converted into a Far Eastern branch of the academy (analogous to the Siberian branch) with an autonomous presidium, it should have a "worthy" material and technical base, and, while the planned new institutes should be built, the existing institutes should be properly equipped and staffed.

Vera Rich

Japanese elders lose their grip on university appointments

Tokyo

JAPAN's Science and Technology Agency has introduced a radically new research system that breaks with the tradition of tenure for academics.

The International Frontier Research System was set up last year at the Science and Technology Agency's Institute of Physical and Chemical Research (RIKEN) near Tokyo with funding of Y1,100 million ($7 million) in the first year rising to Y1,500 million in fiscal year 1987. The programme, scheduled to run for 15 years, consists of 40 researchers in seven teams, split into two groups, "Frontier Materials" and the "Biological Background of Homeostasis". A group to study the artificial brain may be added later.

The rigid system of tenured appointments has long been regarded as a weakness of Japan's universities and research institutes. Professors, once established in a chair, remain entrenched until retirement and enjoy almost dictatorial power over younger researchers who wait patiently in line for a post to be vacated.

Attempts have been made to introduce greater fluidity and flexibility into the system. When the Okazaki institutes for joint university use were set up there was heated debate over whether professorial appointments should be non-tenured. But in the end the only change was a system of adjunct professorships.

The Frontiers system parts from tradition in two respects. About one-third of the researchers, including three of the team leaders, will be non-Japanese. Second, all researchers will be employed under one-year contracts renewable up to a maximum of five years.

The system aims at funding basic research by young scientists that will "sow the seeds of technology in the 21st century", according to Takashi Bito, deputy director of the programme. Patent rights for technology arising out of the research will be shared equally between the Japanese government and the inventor. Frontiers thus in some respects resembles the agency's ERATO programme (see *Nature* **305**, 373; 1983); although Bito says ERATO is more technology-oriented.

The homeostasis group has four teams. Gabriel Gachelin of the Pasteur Institute of France will head the chromosome team, which will study homeostasis and ageing at the gene level. Koichi Chitani, formerly of the University of Washington, heads the ageing process team, which will concentrate its research efforts in the first year on proteins associated with Alzheimer's disease, such as paired helical filaments. Masaki Furuya will resign from Tokyo University to lead research by the plant homeostasis team into phytochromes, and Tomotari Mitsuoka's intestinal flora team at RIKEN will continue to investigate the role of intestinal bacteria in health, nutrition and ageing.

With the rapid 'greying' of Japanese society (see *Nature* **321**, 553; 1986), politicians, academics and pharmaceutical and biotechnology companies are turning their attention to the problems of old age. Researchers from Snow Brand Milk Products, Meiji Milk Products and Nissin Flour Milling Co. will join the intestinal flora team, and special facilities will be set up at RIKEN for raising aged mice and rats.

The three teams of the Frontier Materials group will carry out basic research into the development of new electronic and optical devices. RIKEN's Susuma Namba will head the quantized elements and devices team which will use electron, laser, molecular and ion beams and atomic-layer epitaxy to develop ever smaller electronic devices (such as integrated circuits and Josephson Junctions). Already down to the 100 Angstrom level, Namba works in the realm where quantum effects and electron-wave interference can be observed, and he foresees a new line of engineering emerging from his research, based on electron waves as opposed to electromagnetic radiation.

The non-linear optics and advanced material research team will be headed by Anthony Garito of Pennsylvania University, and will aim at developing non-linear optical devices and two-dimensional superconductors using organic materials, such as methyl nitro aneline. Kevin Ulmer of the Center for Advanced Research for Biotechnology at Maryland State University will lead research by the bioelectronic materials team into electro-and photoactive proteins from which should emerge new biosensors and, perhaps, the biochip. Canon Inc., Ajinomoto Co., Unitika Ltd and other Japanese and US companies are interested in joining the two teams.

But although there seems to be no lack of researchers for Frontier Materials, some of the Homeostasis teams concerned with basic biology are finding it hard to recruit members. Young and talented Japanese researchers, although initially interested, balked at the prospects of having to find a new job after five years. Problems of job security do not arise for the Japanese team leaders themselves — all are at or approaching retirement age, and several hold parallel appointments outside the Frontiers system. A true revolution will have occurred only if younger leaders are appointed when the system undergoes a turnover in staff five years hence.

David Swinbanks

4,000 Chernobyl deaths?

A NEW report from US government estimates that there will be 4,000 excess cancer deaths in Europe and 10,000 in the Soviet Union as a result of the Chernobyl disaster. The report was prepared by the Nuclear Regulatory Commission in the United States. The excess cancers will probably be indistinguishable from the 70 million cancer deaths that are predicted in the European area affected by the fallout over the next 70 years. Chernobyl-induced cancers might be more noticeable in the Soviet Union, where 9.5 million deaths from cancer are otherwise predicted. Estimates of the number of deaths have varied widely; these figures lie at the conservative end of the spectrum. □

Soviet drilling project snag

A US DELEGATION poised to travel to Moscow last weekend to sign a memorandum of understanding making the Soviet Union an official member of the Ocean Drilling Program was ordered not to leave just one day before the scheduled trip. The Defense Department postponed the signing by raising concern about technology transfer through Soviet participation in the project. The Soviet Union would have been the eighth participant in the project, and would have contributed more than $1 million in membership dues for the current fiscal year. □

Cold closes nuclear post

ONE of the three 'nuclear-monitoring' stations installed by a US team in Kazakhstan last year has been put out of action as a result of the severe cold last month. On the night of 16–17 January, the low temperatures at the Bayan-Aul station produced a short circuit which started a fire. Although the monitoring instruments installed in the boreholes remain unharmed, the fire destroyed a building containing all the recording equipment, without which the monitoring programme cannot function. The staff quarters at the site were also destroyed, without loss of life. □

Solar power for Cuba

DR Rosa Elena Simeon, president of the Cuban Academy of sciences, has formally inaugurated Cuba's prototype solar power plant at the Santiago de Cuba solar energy research station.

Cuba has no fossil fuels and its rivers are insufficient for normal hydroelectric generation. Accordingly, as President Fidel Castro stated last July, the construction of thermal power stations will stop, to be replaced by nuclear power, boosted by pumped storage hydroelectricity at peak periods, and some solar power. (Cuba's first nuclear power plant at Gienfugos will have four 4,000 MW Soviet VVER reactors, and will be 'complemented' by a pumped storage plant at Fomento.) □

Australian deserts defended

Sir—Kenneth Mellanby has recently expressed his views on conservation issues in Australia (*Nature* **325**, 112; 1987). By appearing in *Nature*, these views might be given greater credence than they perhaps deserve; although Mellanby is entitled to his opinions, certain statements in his article cannot be left unchallenged.

In late 1986, the federal government did nominate Stage 2 of Kakadu National Park for inscription on the World Heritage List. At the last moment, this nomination was temporarily withdrawn although not as a result of representations from the Northern Territory Government or its lobbyists but as a consequence of legal action taken in the federal court by a mining company.

Mellanby apparently finds much of the landscape of the Northern Territory either "boring" or "dull" — scarcely an objective assessment, nor one universally shared. No doubt many people do find deserts boring; nevertheless, they offer landscapes that others find of peculiar beauty while the biological diversity and the adaptations of the biota to the environment are of considerable interest to both pure and applied scientists. To suggest that eucalypt forests (although technically many of the eucalypt communities in the Northern Territory are woodland) are uniform and dull is to view them with remarkably unperceptive eyes. These ecosystems, which, with the exception of limited areas of eucalypt savanna in Timor and Papua New Guinea, are uniquely Australian and support a great diversity of organisms. A first fleeting glance might suggest a degree of uniformity but anyone who takes the time to look more closely cannot but be impressed by the variety and complexity of these communities. To have described Stage 2 of Kakadu as "the most boring national park in the world" is a greater reflection on the writer than on the area.

Natural properties submitted for inclusion on the World Heritage List are judged against four criteria, of which Professor Mellanby selectively quotes from one only. The full criteria say such properties must:

(i) be outstanding examples representing *the major stages of the earth's evolutionary history*. This category would include sites which represent the major 'eras' of geological history such as 'the age of reptiles' where the development of the planet's natural diversity can well be demonstrated and such as the 'ice age' where early man and his environment underwent major changes; or

(ii) be outstanding examples representing significant ongoing *geological processes, biological evolution and man's interaction with his natural environment*. As distinct from the periods of the Earth's development, this focuses upon ongoing processes in the development of communities of plants and animals, land-forms and marine and fresh water bodies. This category would include for example (a) as geological processes, glaciation and volcanism, (b) as biological evolution, examples of biomes such as tropical rainforests, deserts and tundra, (c) as interaction between man and his natural environment, terraced agricultural landscapes; or

(iii) *contain unique, rare or superlative natural phenomena, formations or features or areas of exceptional natural beauty*, such as superlative examples of the most important ecosystems to man, natural features (for instance, rivers, mountains, waterfalls), spectacles represented by great concentrations of animals, sweeping vistas covered by natural vegetation and exceptional combinations of natural and cultural elements; or

(iv) be *habitats where populations of rare or endangered species of plants and animals still survive*. This category would include those ecosystems in which concentrations of plants and animals of universal interest and significance are found.

(From Nomination form for inclusion of properties on the World Heritage List.)

It is interesting to note that under criterion (ii) "boring" deserts could be considered, while under criterion (iii) Stage 2 of Kakadu certainly offers "sweeping vistas covered by natural vegetation" even if much of it is "dull and uniform" eucalypt communities. I am not aware of the details of the federal government nomination of Kakadu Stage 2. Nomination in itself does not guarantee incription on the list; nominations are subject to a detailed refereeing process as well as examination at the World Heritage Committee itself. When it comes to assessment of natural beauty, then decisions are bound to be subjective; although one would expect the committee to be impartial, its members would not necessarily show the same biases as Mellanby. In judging nominations against other criteria, more objective assessment is possible.

The nomination of Stage 2 of Kakadu has become part of a political agenda thath goes far beyond concern for environmental matters. However, it is not possible to dismiss the case for nomination out of hand as being a mere political ploy.

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Rem overdose

Sir—The communication by R. Russell-Jones on "Cancer risk assessments in light of Chernobyl" (*Nature* **323**, 585; 1986) continues the vapid calculations made following the disaster. It also is technically incorrect as well as misleading. Presumably the 400,000 rem (4,000 Sv) quoted are the product of the mean "dose" (more accurately "dose equivalent") and the number of people (that is, the collective dose equivalent in "man-rem"), and the UK population might over the next five years perhaps receive an average of 10 mrem. This would be a mere two per cent of the natural background dose.

Having been a member of the BEIR III Committee, I know very well that we stated that the effects of background radiation are unknown and that we refused to provide any risk estimates for doses that are less than 10 rem.

Although there are fairly firm scientific reasons for believing that there is proportionality between dose and genetic effects, this so-called 'linear hypothesis' lacks any substantial justification in the case of radiation carcinogenesis and seems in fact most dubious in view of the complexity of the process. It may possibly serve as a crude estimate of the risk attendant to maximum permissible radiation doses but extrapolations by many orders of magnitude, whether made by Russell-Jones or others, are scientifically indefensible.

Harald H. Rossi

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The calchemists

Sir—The increasing use of computers to simulate the properties of chemical entities has led to an unnecessary proliferation of new scientific terms that are unpleasant on the ear. For example, "drug design by computer-based molecular docking", or "molecular dynamics calculations of bulk properties of..." or "computer graphics simulation of solvent–protein interactions" or "computer-generated electrostatic surface representations", are far too latinate to use in normal conversation. I propose that we replace such uses by the historically more suitable term "calchemy" and its derivatives "calchemic", "calchemistry", "biocalchemistry", and begin to raise funds for the formation of new academic departments.

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Joint hunt for missing particles

Cosmologists and high-energy physicists, renowned for radical imaginativeness, seem to have infected solid-state physicists with enthusiasm for the search for dark matter.

ASTROPHYSICISTS and high-energy physicists alike are just now frustrated in their search for missing matter, the former because they have good reason to suppose matter to be present where they cannot see it, the latter because their descriptions require particles not yet found. The realization that both camps may after all be hunting the same entitities has already proved stimulating. It is even more enticing that condensed matter physics, pushed to new limits, may be cheaply used to find the elusive stuff.

Dark matter of one kind or another has been perplexing astrophysicists and cosmologists for some time. Evidence for its existence is still circumstantial — the anomalous orbital behaviour of stars in galaxies and, on a grander scale, theoretical prejudice (otherwise called the inflationary model of the very early Universe) that the mean density of the observable Universe should be about one hundred times that so far detected with visible or other radiation. Of the two principal candidates — light relativistic ('hot') neutrinos and more massive ('cold') particles — the second seems to fit the constraints better.

High-energy physics has its own dilemma. What is called the 'standard model', a combination of quantum chromodynamics (QCD), with its quark-gluon description of strongly interacting particles, and the gauge theory of electromagnetic and weak interactions, has made many successful predictions and is not contradicted by observation. But there remain some 20 free parameters' while the standard model requires the existence of new particles yet to be detected.

One such particle stems from what might be called a minor embarrassment of QCD. Unmodified, the theory would prefer the electric dipole moment of the neutron to be far greater than the tiny upper limit observed. To circumvent this difficulty, a new hidden symmetry has been imposed within the theory which, when broken at the appropriate energy, results in a massive particle called the axion.

This sounds arbitrary and aesthetically unsatisfying, but such shortcomings are outweighed by the explanatory success of QCD. Less experimentally compelling, but at least as appealing to their supporters (though, as the theorist John Ellis once remarked, mother aardvarks find their offspring appealing too), are the supersymmetry theories. Alas, their price for unifying three or all four of the fundamental interactions is a plethora of putative particles.

Here is but one aspect of the cross-fertilization of astronomy and high-energy physics: axions and supersymmetry particles have masses and other properties that make them particularly strong candidates for the cold dark matter, not only pervading the Universe as a whole but also gravitationally bound along with visible matter within galaxies. Galactic dark matter would be streaming through our laboratories but, to be detectable, would have to react sufficiently strongly with ordinary matter by means other than gravity.

Happily, it should. (See, for a review, P.F. Smith, Proceedings of the second CERN-ESO symposium, European Southern Observatory, 1986.) The axion, for example, should interact with a magnetic field to yield a photon. For the expected range of masses, an array of microwave cavities in a five-tesla field could be designed to detect the narrow signature of galactic axions somewhere in the very broad band between 1 and 1,000 GHz.

More widespread interest has been generated by the prospect of catching supersymmetry particles. Not only are the rewards potentially great, but the combination of disciplines involved is entertainingly unusual. In particular, solid state physicists are responding to the novel challenge of how to detect and identify the perturbation in a crystal lattice following the scattering off a nucleus of a dark matter particle. At low temperatures, the recoil of the nucleus generates quantized acoustic waves, or phonons, in the lattice. The trick is then either to detect the ensuing temperature rise or the phonons themselves.

The former is the approach adopted by Bernard Sadoulet and others at Berkeley and by Peter Smith and collaborators at the Rutherford Appleton Laboratory near Oxford. They take advantage of the exceedingly small heat capacity of tiny pure crystals at 10-20 mK: a single 6 keV photon can be detected by the 0·1 per cent temperature rise that it produces.

Different supersymmetry particles (sneutrinos, higgsinos and photinos seem to be the favourites) will give a variety of energy spectra according to the atomic mass and spin of the target nuclei, hence the planned simultaneous use of materials such as silicon, gallium arsenide and lithium bromide. Another signature is the seasonal variation in the particle spectrum caused by the Earth's orbital motion relative to the galactic flux. Such experiments will have to be conducted underground to ensure adequate shielding from cosmic rays.

There are other, perhaps more imaginative and even elegant, ways of detecting a nuclear recoil, such as that developed by A. Drukier and others at the Max Planck Institute at Munich. He proposes to suspend granules of superconducting material within a magnetic field, setting the temperature so that a recoil within a granule would flip it into its resistive state, causing a detectable movement of magnetic flux.

The temperature rise in such small detector crystals (typically of micron thickness) arises from the multiple scattering of phonons off surfaces. Nobody knows for sure what happens if, as might be desirable, the crystal size is increased. Low-energy phonons, at least, experience little scattering within the crystal while the role of surface scattering would diminish, but nobody knows the energy spectrum of phonons generated by a recoil or how all the possible modes will propagate. Furthermore, the scattering properties of surfaces are very sensitive to the presence of small numbers of external atoms of contaminant.

With such uncertainties in mind, ambitious plans have been put forward to detect phonons directly. One being developed by Blas Cabrera and others at Stanford exploits the effect of phonons on a superconductor. If the phonons have sufficient energy they can disrupt the 'Cooper' pairs of electrons which, by means of their interactions with the lattice, otherwise flow freely. The disruption leaves a combination of holes and electrons which can in turn be detected by means of a tunnel junction.

What are the prospects? Even the furthest developed nuclear recoil detectors will take two to three years to become operational. Thereafter, supersymmetry particles may either be found immediately or, if the seasonal signal is required for convincing detection, only after a further year or two. But perhaps they will not be found at all. Agencies being asked to finance these shots in the dark would be right to take the gamble; gamblers elsewhere should bet on the last possibility, but if they are wrong, the excitement would surely compensate for the hole in their pocket.

Philip Campbell

Astronomy

Giant luminous arcs discovered in two clusters of galaxies

Bohdan Paczyński

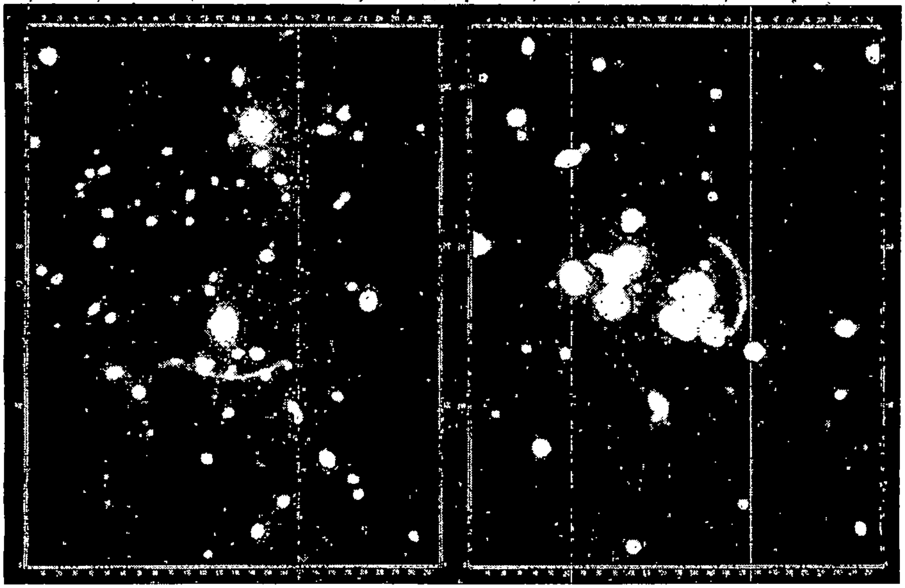
ONE of the highlights of the 169th meeting of the American Astronomical Society, held in Pasadena, California on 4–8 January 1987, was the remarkable discovery[1] reported by R. Lynds (Kitt Peak Observatory) and V. Petrosian (Stanford University): "We announce the existence of a hitherto unknown type of spatially coherent extragalactic structure having, in the two most compelling known examples, the common properties: location in clusters of galaxies, narrow arc-like shape, enormous length, and situation of center of curvature toward both a cD galaxy and the apparent center of gravity of the cluster. The arcs are in excess of 100 kpc in length, have luminosities roughly comparable with those of giant E galaxies, and are distinctly bluer than E galaxies — especially so in one case". The two clusters (see figure) are Abell 370, with a known redshift of 0.373 (ref.2) and 2242-02, for which no redshift has been published. The structures are different from anything yet seen, but nobody doubts that the objects are real.

Lynds and Petrosian found only two cases of the arcs in a sample of 57 clusters. J. Gunn (Princeton University Observatory, PUO) and D. Schneider (Institute for Advanced Study, Princeton, IAS) did not see anything like that in their sample of about 400 clusters of charge-coupled display images obtained on a 200-inch telescope. There are certainly many theoretical speculations about the giant arcs, and I present here a report of the developments as seen from Princeton.

The discovery was first announced on television and in the newspapers, so naturally it was difficult for most astronomers at Princeton to know what was happening. A few days later M. Postman (PUO) returned from Pasadena with more specific news, while a photograph of one arc appeared in *Time* magazine. That was sufficient to trigger several speculations, somewhat predictable in that they may reflect the preoccupations of their originators.

As the arcs are superimposed on clusters of galaxies it is natural to think that they are physically associated with each other. J.P. Ostriker (PUO) suggested that the arcs might be a result of powerful explosions. If the central galaxy of a cluster had an active nucleus some time ago, and released a typical energy of the order of 10^{61}–10^{62} ergs, and there was a lot of cool gas in the cluster of galaxies, then a shock wave would propagate out from the centre of explosion, and it would cool down at a distance of about 100 kpc. Once the spherical shell cooled down it would become gravitationally unstable, and a lot of young stars would be formed. As the arcs do not form complete rings, the gas distribution in the clusters could not be spherically symmetrical. D. Spergel (IAS) proposed that we are seeing a case of galaxy cannibalism, with an arc formed by a galaxy tidally stripped by the cluster.

Over the past few years I have worked on various aspects of gravitational lensing; therefore it was natural for me to propose that the arcs are two new examples of that phenomenon. The possibility that rich clusters of galaxies act as gravitational lenses has been suggested many times[3-6], but so far there is no published and convincing case of such a phenomenon.



Galaxy clusters Abell 370 (left) and 2242-02 (right) showing large arcs. (Photographs courtesy of National Optical Astronomy Observatories, Tucson, Arizona.)

It is well known that if a point source is exactly behind a centre of an axially symmetrical lens, then its image is a circle, sometimes referred to as the Einstein ring. If the lens has an isothermal mass distribution with its components (that is, stars, galaxies, massinos or anything) having a velocity dispersion v^2, then the angular radius of the circle is roughly v^2/c^2, where c is the speed of light. This radius may be as small as 1 arc second if the lens is a medium-sized galaxy with a small velocity dispersion, or as large as 1 arc minute if the lens is a rich cluster of galaxies with a very large velocity dispersion. If the background object is not exactly aligned with the symmetrical lens then we expect to see two arcs on the opposite sides of the lens. A real gravitational lens is not likely to be perfectly symmetrical, and the resulting image of a background galaxy does not have to be a complete ring or a pair of identical arcs on the opposite sides of the lens. Kovner recently presented[7] some theoretical pictures of a gravitationally lensed galaxy, with highly distorted, multiple images. Some of his pictures display somewhat irregular arc-like structures. It is almost certain that by fine-tuning the structure of the gravitational lens it is possible to obtain images with any desired degree of symmetry.

The giant arcs discovered by Lynds and Petrosian are not complete, structureless rings, but rather arcs with noticeable substructure. It is possible that they are images of galaxies located far behind the clusters. The considerable extent of the arcs implies that we are almost exactly in the focus of the lens system, a very unlikely coincidence. On the other hand, the giant arcs are very rare. Taking complex selection criteria and our poor knowledge of the structure of clusters of galaxies into consideration, it is not possible to make a meaningful estimate of the probability of a coincidence. Fortunately, there is a very straightforward prediction of the proposed explanation: the redshifts of giant arcs must be much larger than the redshift of clusters. The arcs are very faint and their redshifts will be very difficult to measure, but once measured they should provide an unambiguous test of the gravitational lens hypothesis.

It is probably premature to speculate more on the basis of rather limited information available. A very important but difficult observation is needed: the very faint spectra of these luminous, giant arcs. If the redshift of arcs turns out to be larger than the redshift of clusters, then the gravitational lens model will be the most likely. If the redshift of arcs turns out to be the same as that of clusters, then some process within the clusters must be responsible for their formation. In this case it may be more difficult to discriminate between the models I have mentioned here. The redshift alone will not be enough; other spectral information will be needed. Considering all the excitement it is somewhat surprising that nobody has suggested that superconducting cosmic strings (another novel topic at Pasadena) are somehow related to the giant arcs. ☐

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Igneous geochemistry

Reading radioactive rock clocks

Ross W. Williams

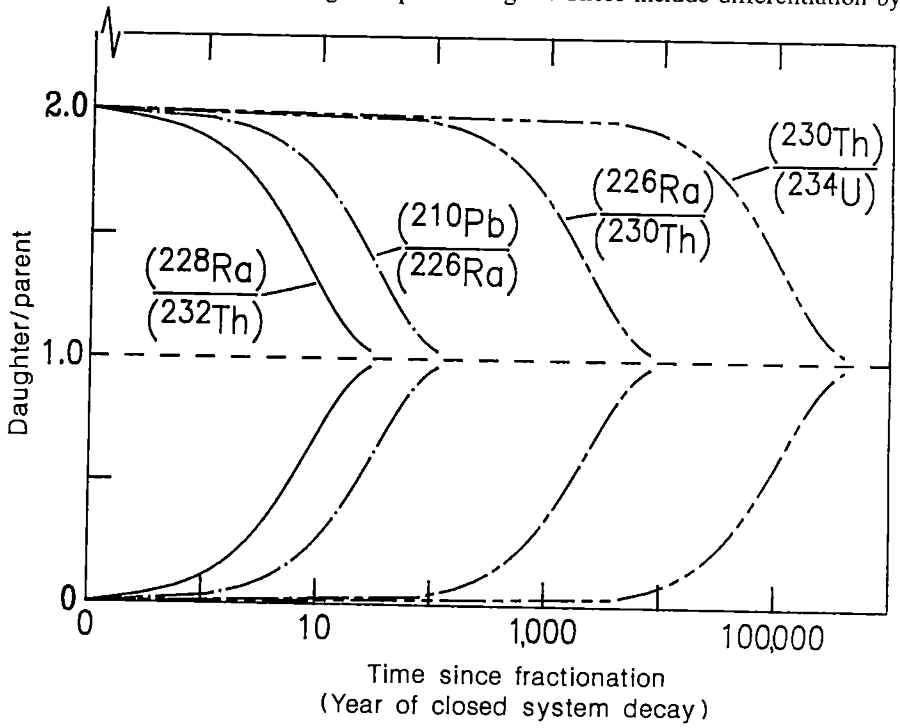
RADIOACTIVE clocks are continuously ticking. In magma source regions, in magma chambers, in minerals crystallized from magmas and after eruption in frozen volcanic effusives, they continue to record time. These clocks are the nuclides of the naturally occurring uranium and thorium radioactive decay series. They are wound by fractionations between the members of the decay series, set when the fractionation process ends and run with characteristic rates determined by the radioactive decay constants of the various isotopes. We are beginning to learn how to read these clocks, as demonstrated by M. Condomines *et al.* on page 607 of this issue[1].

Three naturally occurring radioactive decay series are headed by the very long-lived parents ^{238}U, ^{235}U and ^{232}Th. Each isotope decays through several intermediate steps, represented by nuclides unique to each decay series, to their final daughters ^{206}Pb, ^{207}Pb and ^{208}Pb, respectively, the stable isotopes of lead. When a decay series is in radioactive equilibrium, the decay rate, or activity, of all the intermediate nuclides is equal to that of the parent. Radioactive disequilibrium occurs when, as a result of fractionation, one of the intermediate daughter isotopes is enriched or depleted with respect to its parent. Once the fractionation process ends, the daughter decays or grows towards equilibrium at a rate proportional to its half-life. The figure shows the rates at which different daughter/parent pairs recover to equilibrium (represented by the dashed line at 1.0) after fractionation ends at time t=0.

For almost 30 years, radioactive disequilibria between these nuclide pairs have been used for geochronology by marine scientists. Continuing the analogy with the radioactive clocks, igneous geochemistry has lagged behind marine geochemistry because it is not known how ightly these clocks are wound by igneous processes. That is, we do not yet know the magnitude of the fractionations at t=0 hat can be generated during magma formation, segregation, differentiation, contamination or eruption. Whereas the fractionating phases in the oceans are directly sampled by marine scientists (for example, sediments/sea water, $CaCO_3$/sea water), no direct samples exist of a magma source region, its conduits and chambers, or the country rock and fluids with which it may have chemically interacted. Although potential sources (or sinks) of the decay-series elements in magma systems can be sampled by drilling, or sampled by the magma itself through the presence of inclusions in erupted lava, observed disequilibria have been attributed to particular processes largely by inference.

Uncertainty about which magmatic processes cause fractionation between the decay-series elements is largely the result of the relatively small dataset available for rocks of diverse chemistry and provenance. As radionuclide data for young volcanics are collected, patterns of enrichment in various rock-types begin to emerge. For example, Condomines *et al.*[1] suggest that radium enrichment is correlated with uranium enrichment, which may reflect magma formation in a wet (H_2O–CO_2 fluid) environment. Growing interest in the decay-series nuclides and a promising new analytical development, measurement of ^{230}Th and ^{234}U by mass spectrometry[2], will expand the dataset. Although no mass-spectrometric measurements of ^{230}Th and ^{234}U have been reported for volcanic rocks, their analysis could become routine for researchers with high-resolution, solid-source mass spectrometers equipped with appropriate detectors for small ion currents. Only the most abundant (longest-lived) daughters of the ^{238}U decay chain can be analysed by this method. The activities of the shorter-lived members of the ^{232}Th and ^{238}U decay series will continue to be measured by radiometric counting techniques.

Two of the central questions in igneous geochemistry that study of radioactive disequilibria can help to answer are: what are the rates of magma genesis; and what are the timescales of magma separation and transport? In addition, radionuclide data can help to resolve other questions about processes that occur during the ascent of a magma. These include differentiation by



The curves show the paths that selected daughter/parent activity ratios will follow with time after a fractionation event at t=0. Two sets of initial conditions are shown for each nuclide pair: (1) a twofold enrichment of the daughter over the parent (upper traces); and (2) no daughter present at t=0 (lower traces). The time required to reach equilibrium is proportional to the half-lives of the daughters. For each pair, the curves end at t=5 times the daughter's half-life (~ 97 % ingrown).

solid/liquid, liquid/liquid or liquid/vapour fractionation mechanisms; and contamination, which can include mixing with other magmas and assimilation of wall-rock or foreign fluids.

This research promises to increase our understanding of the physiochemical nature of igneous processes, as well as the timescales over which they operate. Researchers have begun to use the radioactive clocks to see into the mantle of the Earth where magmas are generated. D. McKenzie[3], interpreting the $(^{230}\mathrm{Th})/(^{238}\mathrm{U})$ data for mid-ocean ridge basalt[4,5] by a dynamical melting model for genesis of this basalt, has suggested that "the melting occurs over a time long compared with the decay time of ^{230}Th, and that less than 2 per cent of the rock is molten at any time". Although an important contribution, McKenzie's interpretation can be challenged because of the restricted values for the solid/liquid distribution coefficients of uranium and thorium which he considered. As these distribution coefficients are more precisely determined, however, decay-series activity data will help to constrain partial melting processes and their relative timescales.

Integrating radionuclide data with other isotope and trace-element data is a powerful method for investigating igneous processes that cannot be fully understood by any one of these methods separately. The radioactive clocks will therefore become an indispensible tool for igneous petrologists involved in the study of magma dynamics □

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Molecular medicine

Oncogenes in atherosclerosis

James Scott

ATHEROSCLEROSIS, a disorder of large arteries that develops slowly over many years, is the cause of coronary heart disease and cerebrovascular disease. The lesions of atherosclerosis occur in the innermost layer of the affected arteries and consist of plaques of proliferated smooth muscle cells infiltrated with macrophages and embedded in a matrix of basement membrane, proteoglycan and connective tissue. Many of the cells in this lesion have taken up cholesteryl esters derived from low-density lipoprotein (LDL) and their cytoplasm has a characteristic foamy appearance. The most popular theory[1] for the pathogenesis of these atherosclerotic plaques is that they develop as a consequence of chronic cycles of vascular wall injury and repair, and that injury is exacerbated by risk factors for atherosclerosis such as hypercholesterolaemia, hypertension and smoking cigarettes. An alternative and less-fashionable view[2,3], based on the observation that atherosclerotic plaques have monoclonal character for isoenzymes of glucose-6-phosphate dehydrogenase (G-6-PD), is that each plaque represents a benign neoplasm derived from a cell that has been transformed by viruses or chemicals. The recent demonstration[4] by Penn and colleagues that human atherosclerotic plaques contain active oncogenes gives new impetus to this 'monoclonal' hypothesis.

Pathological studies in man show that development of the fatty, fibrous atherosclerotic plaque is preceded by the fatty streak, a subendothelial accumulation of cholesterol-engorged macrophages and smooth muscle cells. Animals that are fed cholesterol show a similar progression from fatty streak to fibrous plaque, with macrophage adherence to and infiltration of the endothelium being significant early events[1]. Later, there is endothelial retraction over fatty streaks and formation of platelet thrombus; ultimately smooth muscle cells and matrix components accumulate to result in a fibrous plaque. Each cellular element involved in the pathogenesis of the atherosclerotic plaque harbours platelet-derived growth factor (PDGF), the major mitogen for vascular smooth muscle cells[1,5]. PDGF is a basic protein of relative molecular mass 30,000 with two disulphide-linked chains (A and B) and is active as a homodimer of A or B and as a heterodimer of A and B chains. It is also a chemoattractant for smooth muscle cells and stimulates the secretion matrix components from them. The most abundant source of PDGF is the platelet α-granule, the constituents of which are released during platelet adherence to damaged vascular endothelia. Apart from platelets, it has recently been shown[5,6] that PDGF B chain is produced by macrophages and endothelial cells and that the A chain is secreted by smooth muscle cells in tissue culture. Platelets and macrophages also contain factors that effect endothelial cell mitosis including α- and β-transforming growth factors and fibroblast growth factors[1,6]. Moreover, smooth muscle cells secrete insulin-like growth factor-I which augments the mitogenic effect of PDGF on these cells[6]. Taken together, these observations suggest that PDGF, produced by platelets in response to endothelial injury and by macrophages and arterial wall cells in response to more subtle damage, leads to the recruitment and proliferation of smooth muscle cells and to the secretion of the matrix components that cause the atherosclerotic plaque[1].

It is well known that human atherosclerotic plaques have monoclonal character for isoenzymes of G-6-PD (refs 2,7,8). The gene encoding G-6-PD resides on the X chromosome so that, in accord with the Lyon hypothesis[9], one copy is inactivated randomly during development in females. As a consequence most tissues form a mosaic of patches derived from cells in which one or other X chromosome has been inactivated. The patches are generally small so that representative samples of tissue in heterozygous individuals will contain both isoenzymes of G-6-PD. The finding of a high frequency[2,7] of monoclonality for G-6-PD in atherosclerotic plaques led to the suggestion that the plaque is a primary benign neoplasm[2]. But Thomas *et al.*[8] found the monoclonal character for G-6-PD in only 30 per cent of the many lesions they investigated. The explanations they considered include secondary selection of smooth muscle cells that have undergone mutation, producing a selective growth advantage; the phenotypic selection of cells containing one or other of the isoenzymes of G-6-PD resulting from the remote presence on the X chromosome of an allele that confers advantage; or more simply that the monoclonal character of plaques is caused by sampling of plaques originating from a single patch of the aortic mosaic.

The work of Penn and colleagues[4] provides further support for the monoclonal hypothesis. They demonstrate the presence of an active human oncogene in plaques by transformation of cultured mouse fibroblast NIH 3T3 cells with transfected DNA from plaques. The transformed cells could establish tumours in 'nude' mice, but unlike the aggressive growth produced by the *ras*, *met* and *neu* oncogenes, of which *ras* was excluded by hybridization, the tumours produced by plaque DNA have a slow growth rate and low tumour incidence. No transforming potential is elicited from normal arterial wall DNA. These studies therefore implicate mutation leading to activation of one or more dominantly active oncogenes in the cells that make up the atherosclerotic plaque — a process demonstrated previously only in cancer cells.

If the observations of Penn *et al.* can be confirmed, they raise important questions concerning the stage in the pathogenesis of the atherosclerotic plaque at which oncogene activation occurs and the cell type that is transformed. Although they did not examine fatty streaks, these lesions do not apparently show monoclonality[7]. The fatty streak does, however, provide an environment with significant mutagenic potential in which oncogene activation might occur and lead to plaque development. Chronic inflammation certainly predisposes to neoplasia and transformation can be produced by human phagocytes stimulated to produce lipid peroxides, reactive oxygen intermediates[10,11]. In addition, macrophages and endothelial cells modify LDL by free-radical attack with the peroxides[12,13] and the core of LDL may act as a sink for lipid-soluble mutagens. Significant amounts of modified LDL may be taken up by macrophages and smooth muscle cells during generation of foam cells, predisposing them to transformation.

What is the active oncogene in the atherosclerotic plaque? PDGF is an obvious candidate because its B-chain gene is almost identical to the transforming gene of the simian sarcoma virus[1,6]. This virus transforms many PDGF-responsive cells in tissue culture, including smooth muscle cells, fibroblasts and NIH 3T3 cells, and the phenotype of virus-transformed cells is relatively benign, being intermediate between that of normal cells and that produced by Kirsten mouse sarcoma virus. Many cells transformed in culture by agents other than simian sarcoma virus or obtained from naturally occurring tumours produce a PDGF-like protein. It is not clear whether PDGF produced in these ways contributes to the neoplastic process by an autocrine mechanism or whether it has a paracrine effect that recruits mesenchymal cells into the tumour mass. But these observations may be important in relation to atherosclerotic plaque formation. If proto-oncogene activation leads to increased secretion of A and/or B chains as either a primary or a secondary mechanism, there would certainly be increased smooth muscle growth within the arterial wall. ☐

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X-ray scattering

A new probe of protein dynamics

Peter Artymiuk

THE importance of the dynamic aspects of the behaviour of biological macromolecules is now widely recognized. In many areas of molecular biology, such as muscle contraction or conformational changes in allosteric proteins, motion is self-evidently crucial. Again, in facilitating such processes as the access of ligands to binding sites on proteins, or in accommodating the change in protein and substrate geometry associated with the execution of an enzymic reaction, coordinated molecular motion is often essential. Knowledge of dynamic processes in proteins initially came from spectroscopic methods such as fluorescence quenching and nuclear magnetic resonance, and has been increased by theoretical molecular-dynamics simulations[1]. The spectroscopic methods give very local dynamic information concerning only a few of the amino-acid residues in a protein. More recently, analyses[2,3] of the isotropic atomic temperature factors, obtained from crystallographic refinement against the Bragg diffraction intensities from protein crystals, have provided an overall view of the amplitudes of displacements of atoms throughout complete protein structures. A further step towards the understanding of protein dynamics in the crystal is described on page 643 of this issue[4], where Doucet and Benoit report on one aspect of the diffuse, non-Bragg scattering of X-rays from crystals of lysozyme.

Two types of X-ray diffuse scattering can be distinguished[5]. The first is thermal diffuse scattering, which concerns modes of vibration and elasticity in the crystal. It arises from correlated displacements of atoms throughout the crystal, and is manifested as diffuse spots around the positions of the Bragg peaks. In principle, the positions, shape and intensities of these thermal diffuse maxima contain information about the direction, location and extent of correlated displacements. Extracting this information would provide invaluable data about concerted motions of groups of atoms within protein molecules. The second kind of diffuse scattering results from disorder and is attributable to crystal imperfections.

It is this latter aspect of the diffuse scatter that concerns Doucet and Benoit. These authors demonstrate[4] the existence of correlated rigid-body displacements of groups of lysozyme molecules in a crystal. Clearly, these displacements are just a function of the particular crystal form of lysozyme studied, and are not of biological significance. But because the rewards of more complete analyses of diffuse scattering would be so great, the emergence of methods for measuring and modelling the diffuse scattering is very important. It is to be hoped that the methods used by Doucet and Benoit will be extended to include thermal diffuse scattering, perhaps through the use of molecular-dynamics simulations[1]. One example of the analysis of X-ray diffuse scattering from protein crystals to yield biologically pertinent data already exists: Phillips *et al.*[6] have investigated diffuse scattering from tropomyosin crystals and attempted to relate the crystalline disorder to the movement of tropomyosin during regulation of muscle contraction.

In principle, the information that could be obtained from diffuse scattering would substantially augment that obtainable from crystallographic temperature factors: there would be the possibility of describing large displacements of whole subunits or domains in proteins; the presence or absence of cooperativity between different displacements could be established; and the directionality of displacements could be defined without the requirement, necessary for anisotropic temperature-factor refinement, of unusually strong diffraction at high angles.

There is, however, a major deficiency in the information about dynamics available from both X-ray diffraction and X-ray diffuse scattering: both supply time- and space-averaged accounts of the distribution of matter within the crystals and give no information about the timescales of the displacements they detect. Indeed, the displacements discovered may not be dynamic at all, but may result from static disorder in the crystal. There have been attempts to discriminate between static and dynamic disorder by doing experiments at different temperatures[7], but because dynamic disorder can 'freeze' into static disorder at low temperature, such studies may not be conclusive. In thermal diffuse scattering, authentic dynamic effects cause a tiny change in the wavelength of the scattered X-ray as energy is inelastically transferred to quantized lattice vibrations (phonons) in the lattice, but this change is too small to be measured. Inelastic neutron scattering has been used to demonstrate[8] the dynamic origins of thermal diffuse scattering in β-quartz, but has yet to fulfill its promise in probing protein dynamics. At this stage in our understanding of motion in biological macromolecules, however, data on conformational variations, whether they happen to be static or dynamic in the crystal, are valuable indicators of the inherent flexibility of protein molecules.

But does the behaviour of protein molecules inside a crystal have any relevance to the dynamic properties of protein molecules in solution? Petsko and Ringe[9] have reviewed this question and the evidence (which includes studies of enzymic catalysis and of hydrogen–deuterium exchange in the crystal) suggests that the answer is often yes. The reason is that typically 50 per cent of the volume of a protein crystal consists of wide expanses of the solvent of crystallization (usually bulk water or an aqueous salt solution), with relatively few actual contacts between protein molecules. Although crystal contacts can have a damping effort on the atomic displacements[10], it is frequently found that large-scale conformational variability can be accommodated within protein crystals. In the crystals of immunoglobulin IgG, for example[11], the entire F_c domain (a region of relative molecular mass 50,000) is disordered and cannot be seen in the electron-density maps. Such cases of disorder must be related to the inherent flexibilities of the protein molecules concerned, which in turn may be related to their function.

In the past few years, the use of high-intensity synchrotron radiation for crystallographic data collection has shown that many protein crystals exhibit diffuse scattering maxima. The study of these potentially very informative adjuncts to diffraction patterns is only just beginning. Nevertheless, it is to be hoped that before long, analysis of X-ray diffuse scattering from such crystals will reveal much more about cooperative motions and conformational variability in protein molecules. ☐

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Reinhold Benesch (1919 – 1986)

SOME people are said to be accident-prone. Reinhold Benesch, who died in New York on 30 December 1986, was discovery-prone. His first piece of research was a contribution to the British war effort. When asked to develop a way of packing eggs for the troops more tightly, he fed hens with sulfanilamide, which inhibited the enzyme carbonic anhydrase that is essential for calcification of their eggshells; the shell-less 'floppy' eggs could be packed like matchboxes. Benesch never told me whether the army adopted his invention. Once he sent me a reprint of a paper entitled *Homos and Heteros Among the Hemos* inscribed "With cordial greeting R^2B^2". The paper was about homo- and hetero-oligomers of haemoglobin and the signature symbolized the 'heterodimer' Ruth and Reinhold Benesch. They got married in England in 1946, emigrated to the United States in 1947 and for the past 26 years worked together at the Columbia College of Physicians and Surgeons. They became the joint authors of 125 papers, all but 13 on haemoglobin. Ruth's contribution to them is inseparable from Reinhold's.

Oxygen equilibrium curves of purified human haemoglobin solutions differ from those of haemolysates. This made the Cambridge physiologist Joseph Barcroft suspect as early as 1921 the presence of a "third substance ... which forms an integral part of the oxygen haemoglobin complex". Years later, it also made his pupil F.J.W. Roughton dismiss my X-ray work on pure haemoglobin crystals as physiologically irrelevant. In 1967 the Benesches discovered that Barcroft's third substance is 2,3-diphosphoglycerate (DPG), long known to be present in erythrocytes in equimolar proportion to haemoglobin. That discovery opened a new era in the physiology of respiratory carriage. Some of the best among the ensuing flood of papers are those by the Benesches themselves. They demonstrated that one mole of DPG combines with one mole of deoxy-, but not with oxyhaemoglobin and that DPG lowers the oxygen affinity in a physiologically advantageous way. They determined the thermodynamics of DPG binding; its influence on the Bohr effect and its interactions with other solutes; they also foreshadowed its location in the central cavity between the β-chains which A. Arnone later determined by X-ray analysis. Subsequent work by several investigators indicated that DPG also binds weakly to oxyhaemoglobin and that its more powerful analogue inositol hexaphosphate (IHP) binds to oxyhaemoglobin quite strongly. Judging by chemical evidence, DPG and IHP appeared to bind to the same residues as in deoxyhaemoglobin, even though the cleft where they bind is closed in oxyhaemoglobin. I tried to solve that paradox by X-ray analysis, but drew a blank. The Benesches invented an ingenious method for measuring the dissociation constants of weakly bound ligands and used it to demonstrate that DPG and ATP, and by implication also IHP, bind, not to oxyhaemoglobin tetramers, but to $\alpha\beta$-dimers, where the residues that are tucked away in the cleft of the tetramer are exposed. The satisfying answer to this long-standing puzzle forms the subject of the Benesches' last, and perhaps most elegant, joint paper.

On several other occasions their work brought light where there had been darkness. The oxygen equilibrium curves of human haemoglobin measured by different workers used to be inconsistent, because their solutions contained variable amounts of DPG and they used anionic buffers which bound to deoxyhaemoglobin and reduced the oxygen affinity in varying degrees. The Benesches developed methods of stripping away the DPG and introduced bis Tris buffer which has a *p*K of 6.5 and is mostly uncharged at physiological *p*H, so that it is not bound by deoxyhaemoglobin. Only since then have oxygen equilibria become reproducible. They started their first work on haemoglobin in response to reports that its SH groups contribute to the Bohr effect. The Benesches disproved this, even though they found that the SH reagent *N*-ethylmaleimide halves the alkaline Bohr effect. That discovery led me to examine the *N*-ethylmaleimide derivative by X-ray analysis, which revealed that this reagent inhibits formation of a salt bridge formed by the carboxy-terminal histidines of the β-chains and thus led to the first interpretation of a physiological function of haemoglobin on a stereochemical basis. The paper on homos and heteros among the hemos demonstrated that tetramers of β- and γ-chains, which undergo no change in quaternary structure, bind oxygen non-cooperatively, thus proving that a heterodimer undergoing a structural transition is essential for cooperativity.

On loss of oxygen, sickle-cell haemoglobin polymerizes into long fibres. Knowledge of the contacts between the molecules in the fibres would facilitate the design of anti-sickling drugs. The Benesches explored these contacts by making hybrids of β-chains carrying the sickle-cell mutation with a variety of mutant α-chains. They found their effects to be highly specific: some mutations had no influence on the solubility of deoxyhaemoglobin S; some raised it; one lowered it and changed the fibre geometry. The results enabled them to map out a hitherto unrecognized contact area. Recently they discovered that folate, in its poly-γ-glutamate complex, is carried in the blood by haemoglobin to which it binds at the same site as DPG.

Reinhold Benesch was a first-class biochemist and also an accomplished wit and comedian who brought laughter to the dreariest of scientific meetings. His inventiveness and originality shone in his papers and in discussion; his wide literary knowledge and *joie de vivre* made him a delightful companion on non-scientific occasions.

Max Perutz

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Atomic physics

New patterns in atomic spectra

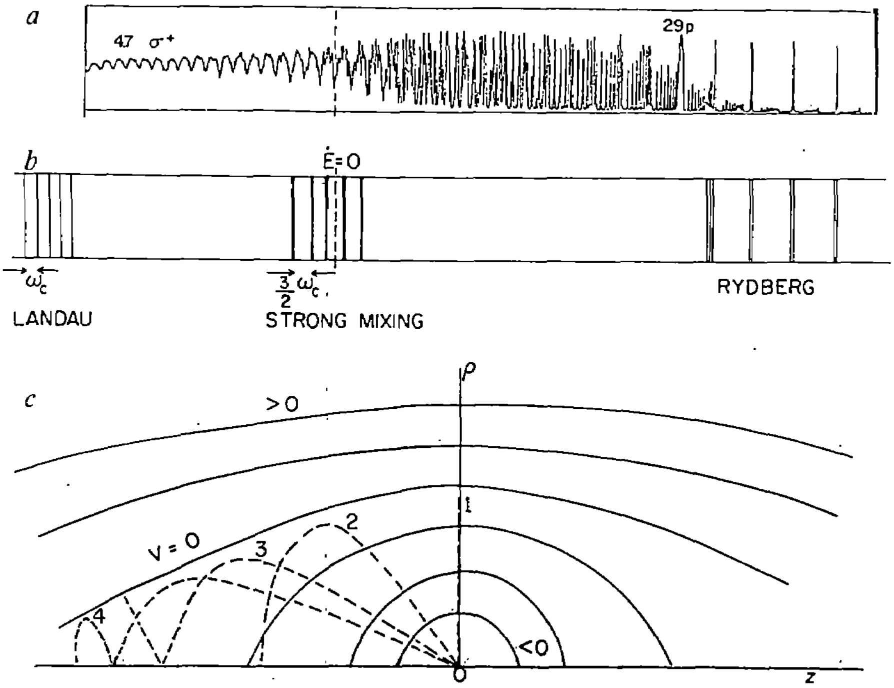
A. R. P. Rau

CAN there still be anything new in the problem of a hydrogen atom in a uniform magnetic field? Most physicists are likely to say no and be surprised that this seemingly simple problem is not yet fully understood and that unexpected and qualitatively new phenomena continue to emerge. Recent experiments[1,2] by a group at the University of Bielefeld provide the latest twist to a fascinating story that has been developing over the past fifteen years[3-5]. The story is a good example of how new patterns and structures, whose understanding poses non-trivial questions, can emerge from combining two well-understood forces. This simple atomic system seems to be rich in its implications for the general study of processes such as non-separable and non-integrable classical and quantum mechanics, and the nature and onset of chaos.

The motion of an electron in the Coulomb field ($-e^2/r$) of a positive charge or in the diamagnetic potential ($\frac{1}{2}B^2\rho^2$, where $\rho^2 = x^2 + y^2$) of a uniform magnetic field B (along the z-direction) is a familiar one when either field acts alone. The Coulomb problem has an infinite Rydberg progression of bound-state energy levels that terminate at the edge $E = 0$ of the continuum; the higher energy levels ($E \leqslant 0$) are highly degenerate. The magnetic problem, on the other hand, has an infinity of equally spaced energy levels for the motion in x and y (the Landau spectrum, with spacing equal to $\hbar\omega_c$, where ω_c is the cyclotron frequency and $\hbar$ is Planck's constant) and free motion along z. Once again each Landau level is highly degenerate. When both fields act together, the situation is again familiar when the magnetic field is weaker than the Coulomb field. Perturbation theory then applies for the diamagnetic interaction or for the linear coupling of the magnetic moment of the electron to the magnetic field (Zeeman and Paschen–Back effects). The spectrum is a Rydberg pattern with some sub-structure imposed by the magnetic field on each energy level. Similarly, in the past fifteen years, in dealing with atomic structure on neutron stars (believed to have large magnetic fields, $B \sim 2 \times 10^8$ tesla), there have been extensive studies[6] of the regime when the diamagnetic potential is dominant and the Coulomb field secondary. Again, standard techniques apply and the spectrum can basically be described as equally spaced Landau levels (spacing ~ 10 keV) with sub-structure caused by the Coulomb potential.

But what happens in between, when neither field can be considered perturbative and both terms in the Coulomb–diamagnetic potential, $V(\rho,z) = (-e^2/r) + \frac{1}{2}B^2\rho^2$ (where B is in units of 4.7×10^5 tesla), are equal. For any value of B, this situation will obtain around $E = 0$. Starting with the basis of eigenstates of either field alone, an infinite number of states are mixed together by the full potential. One may then expect no simple regularities or structures in this "strongly mixed"[3] region of the spectrum. Yet in 1968 the first experimental results from this region belied this expectation. The figure (*a*) shows an absorption spectrum[7] of the principal series in strontium in a field of B = 4.7 tesla. The most striking feature of this and subsequent experiments with other atoms is the pattern of broad, equally spaced resonances around $E = 0$. Regardless of the atom, the spacing between resonances is $(3/2)\hbar\omega_c$.

Therefore, as shown in *b*, the problem of combined Coulomb and diamagnetic motions seems to have three, not two, regimes with characteristic signatures. Complementing the two signatures of Coulomb and Landau patterns, when one or the other field is dominant, is the equally characteristic signature of the strong-mixing regime[3]. A full understanding of the origins of a simple pattern in a highly non-perturbative regime and the generality of this result (similar results obtain for strong mixing of Coulomb and electric fields[3]) are still open questions. The new results[1,2], the first experiments with hydrogen, hold another surprise. As well as the $(3/2)\hbar\omega_c$ resonances, there are other families of equally spaced resonances with other spacings.



a, Absorption spectrum of strontium with $\sigma+$ (m=1) polarization in a 4.7-tesla magnetic field (from ref. 7). *b*, Schematic drawing of *a* to emphasize three characteristic regimes. *c*, Equipotentials of Coulomb–diamagnetic potential. The numbered paths correspond to different modes of quantization around $E = 0$.

The Coulomb–diamagnetic potential $V(\rho,z)$ is non-separable and not exactly solvable. A partial understanding of the experimental observations can be gained from the equipotental lines sketched in *c*. After the initial experiments, it was realized[8,9] that in considering the motion of an electron on this equipotential surface, if one envisages motion along the ρ-axis, then for every energy E (even $E > 0$) this motion is bounded. The electron has both ρ- and z- motions; the two are coupled by the Coulomb term in $V(\rho,z)$ so that for any $E > 0$ the latter motion will eventually lead to escape. But, application of a semi-classical quantization condition for the path 1 gives[3,8,9] for $E \sim 0$ a set of energy values with equal spacing of $(3/2)\hbar\omega_c$. The new spacings seen in the hydrogen experiment have now been similarly correlated[2] with the other paths in *c* through both classical trajectory calculations for recurrence times following successive reflections from the $V = 0$ and $\rho = 0$ lines, and corresponding semi-classical quantization along the paths shown[2,10]. One can expect decreasing intensity with increasing length of paths. This fits with the $(3/2)\hbar\omega_c$ spacing being dominant in nearly all experiments to date, except one[1] when the vanishing of the wavefunction along the ρ-axis disfavours it. It is also clear from c, as noted by the Bielefeld group[2], that for the higher-numbered paths, the higher bounces at large z take place mainly under the diamagnetic influence, the Coulomb term being insignificant; therefore, successive recurrence times are staggered by the cyclotron period $T_c = 2\pi/\omega_c$.

There is as yet little progress in going beyond such simple one-dimensional semi-classical calculations for energy spacings. A fuller understanding of the quantum mechanics of the non-separable potential still eludes us. Intensities and widths of the observed strong-mixing patterns remain unexplained.

The Coulomb–diamagnetic problem has also become of interest as a real physical system that is amenable to experiments in which questions of classical and quantum chaos can be studied. Classical trajectory calculations show a passage from regular patterns in phase space to a random chaotic distribution as the $E = 0$ limit is approached from negative energies. Similarly, large-scale numerical diagonalizations of $V(\rho,z)$ in a basis of Coulomb wavefunctions show that the spacing between eigenvalues has a Poisson distribution so long as $B^2/|E|^3$ is small but changes to a Wigner distribution as this parameter becomes large[12,13]. Given the infinite pile-up of Rydberg levels at $E = 0$, all such calculations have perforce stopped a little short of the ionization limit.

Clearly, much is still unknown about this simple but rich atomic system. There is already an indication of further surprises in store because the hydrogen experiment shows other spacings that cannot be correlated with the sequence of paths in c. But it is also clear that more experimental and theoretical understanding of this fascinating system of hydrogen in a magnetic field will have wider application for the physics of non-separable potentials. Just as the development of perturbation theory through the study of the Zeeman and Stark effects has contributed tools for general theoretical physics, so also a fuller elucidation of strong mixing in this system is likely to be of interest elsewhere in physics. ☐

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Neurobiology

Disappearing developing cells

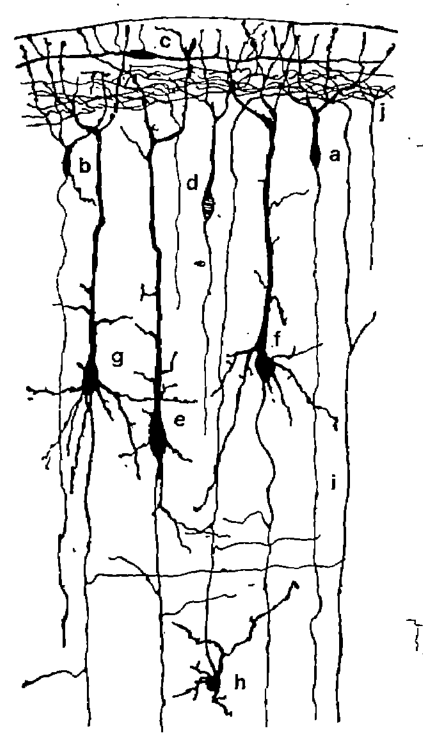
R. W. Guillery and H.P. Killackey

THE early formation of nerve cells and connections, produced in excess of adult needs and made with many 'errors', has over the past 30 years become an acceptable concept to neurobiologists[1]. Such regressive phenomena are believed to contribute to the formation of an accurately connected nervous system. The possibly related phenomenon of transient nerve cells, which are present in early development but have no adult counterparts, has been raised in the past, but Chun, Nakamura and Shatz on page 617 of this issue[2] report new evidence for a population of cortical neurons that establishes synaptic connections and subsequently disappears. This observation suggests that regressive phenomena such as cell death are involved in processes other than error correction during normal neuronal development.

The population of cortical cells reported by Chun *et al.* lies in the cortical subplate; the cells are born early, have local dendrites and longer axons, receive synaptic connections, develop putative transmitters and then largely disappear during early postnatal development. These cells probably correspond to the Martinotti cells of deep cortical layers (see figure) and the Cajal–Retzius cells of superficial cortical layers, both of which are also formed very early. The disappearance of these cells has long been suspected but it has never before been clear whether they became modified into other cell types or degenerated; nor was it known whether at early developmental stages they had established synaptic connections. By selectively labelling the oldest cortical nerve cells using pulse labels of radioactive thymidine, Chun *et al.* now show that the earliest-formed cortical cells fail to survive even though they develop all the neural characteristics listed above.

What are these neurons doing? They are found before the major cell layers (II–V) of the cortex have developed, and occur simultaneously with the entry of the thalamocortical and interhemispheric afferents into the cortical subplate region[3]. Their axons pass to deep cerebral structures and to the opposite hemisphere[2]. Although the cells are immunoreactive for neuropeptides it is not yet known whether these peptides are transported down the axons or whether the axons establish synaptic contacts. Electron-microscope evidence, however, shows that the cells do receive synaptic contacts.

If the cells turn out to be electrically active at this early stage then one can suppose that the temporary impulse traffic is involved in the further development of cortical and subcortical circuitry; the cells are unlikely to play a role in the transmission of sensory or motor functions of the organism at this stage. If they are not discharging impulses then they are probably concerned with local interactions at each end of the long axonal process; these interactions may be temporary trophic effects.



Ramon Y Cajal's drawing[8] of cortex from a four-day-old mouse. This Golgi preparation shows the Martinotti cells (h) and the Cajal – Retzius cells (c) in the cortex at a stage later than that described by Chun *et al.*[2], after the intermediate cell layers have developed. See text for further details.

One such interaction could be the maintenance of a cell population which later acquires a different postsynaptic target, such as the thalamic cells whose axons have reached the cortex at this stage but are 'waiting' in the subplate. The transient cells may provide a temporary target for these axons, because the cortical cells that they will eventually innervate do not develop until much later. The evidence that the transient cells receive contacts with all the morphological appearance of an adult synapse may make morphologists reluctant to think of the cells as electrically inactive. If nerve cells can make contacts that look like synapses, at which no electrical interaction occurs, then the morphologist is bound to question the significance of the features that are classically considered diagnostic of a synapse.

The transient cells may also serve as tropic markers or 'guideposts' providing a telencephalic equivalent to the guidepost cells defined for developing pathways in other nervous systems[4]. On either hypothesis, however, the function of the axons remains obscure. The long intercerebral pathway is particularly difficult to understand. If it provides a precursor of callosal pathways, why are such precursors needed?

A further possibility is that the transient cortical cells represent the exploitation during mammalian development of cells surviving in the adult of pre-mammalian forms[5]. This would account for the origin, but not for the function, of the cells.

The sequence of developmental events producing the circuitry of the adult brain is extraordinarily difficult to understand. Even where the complexities of the adult are understood (there are few such regions: the cerebral cortex is certainly not one) there are no clear ideas about the ways in which specific patterns of connections are formed. Guidepost cells, substrate pathways[6] or temporary neural projections that are obliterated or pruned and corrected[7], provide a conceptual crutch for understanding how complex patterns of connectivity could be formed. But temporary measures for carrying instructions that eventually make a brain are like messengers arriving at a general's headquarters — it is useful to see them arriving, and perhaps even to identify them, the course of their journey and their effect on subsequent events, but the outcome of the battle will be most clearly understood if the nature of the message and the instructions given to the messenger (not necessarily distinguishable) are understood. On these subjects developmental neurobiologists must, as yet, remain silent. □

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100 years ago

BUBBLES in ice are familiar; but their arrangement and progressive development in the process of freezing-over present some points which I do not think have been generally observed. The following facts must be noticed: (1) Ice over deep water invariably contains fewer bubbles of included air and gas than ice formed over shallow water. (2) The upper portion of a coating of ice invariably contains less included air than its lower portion. (3) There is more included air ice formed over water in a small vessel than ice formed over a large body. (4) There is more included air in an entirely frozen mass of ice than in surface ice from a partly frozen vessel. (5) In freezing separately the water from which the first frozen coat of ice had been removed, the ice contained a much larger proportion of included air than either the surface ice or the ice obtained from entirely freezing a body of water.

From *Nature* **35,** 325; 3 February 1887.

Topology

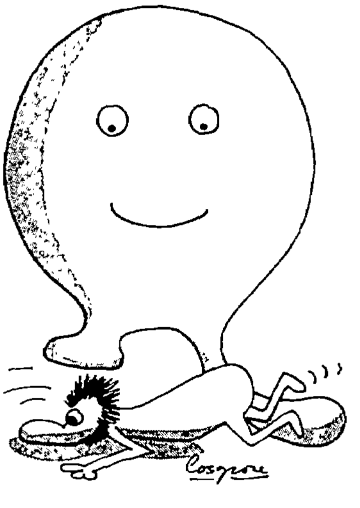
The three-sphere strikes back

Ian Stewart

ALMOST a year ago I reported in a News and Views article (*Nature* **320**, 217–218; 1986) the announcement, by Eduardo Rêgo and Colin Rourke, of a proof of the Poincaré conjecture — one of the major unsolved problems of topology. At first, the mathematical community regarded the claim with scepticism. There were good reasons for this. Proofs have been announced before, and retracted soon after, by many distinguished mathematicians. Some thought that Rêgo and Rourke's methods were not novel enough. Their proof was also long and complicated, difficult to grasp in its entirety and rather sketchy in places. But, for the time being, the sceptics are right.

During the first year, the proof was subjected to thorough scrutiny by the mathematical community. Against most predictions, it held up well until early November 1986. Then Rourke gave a series of seminars to an audience of experts at the University of California at Berkeley, whose objective was to settle the matter once and for all. During a routine discussion, Rourke realized there was a mistake, and pointed it out to the audience. It is in the nature of mathematics that a defective proof is no proof at all, so the original announcement was wrong. But the story may not be over yet.

The conjecture is about manifolds, which are among the most important objects in topology. Manifolds are spaces which 'in the small' resemble ordinary euclidean space, but which, 'in the large', can bend round upon themselves, twist up in complicated ways, have holes and so on. In topology, two manifolds are considered to be the same if one can be obtained from the other by a continuous deformation. The surface of a sphere, and that of a torus (or doughnut), are two-dimensional manifolds, but they are topologically distinct because the torus has a hole whereas the sphere does not.



One way to detect the hole in the torus is to thread a loop through it. This loop cannot be shrunk continuously down to a single point while remaining on the surface of the torus. In contrast, on the sphere, every loop can be so shrunk. During the nineteenth century, mathematicians were able to find all possible topological types of two-dimensional manifolds. One consequence is that the only two-dimensional manifold on which all loops can be shrunk to a point is a sphere.

Poincaré was interested in the next step: three-dimensional manifolds, which are much more complicated and at one of the great open frontiers of topology. Even the simplest questions remain unanswered, and the most basic is Poincaré's.

The natural three-dimensional generalization of the two-dimensional sphere is what Poincaré called a hypersphere, and is now called a three-sphere. In the hypersphere, as in the ordinary sphere, every loop can be shrunk to a point. Poincaré asked whether this property completely characterizes the hypersphere. If every loop in a given three-dimensional manifold can be shrunk to a point, must that manifold be topologically a hypersphere? Poincaré asked the question in 1904, and nobody knew how to answer it, although it was widely assumed that the answer must be yes. The problem was still open when Rêgo and Rourke announced their proof.

Their method is technically complicated, but based on familiar ideas (some, indeed, going back to the time of Poincaré). They start with a description of a three-dimensional manifold in terms of a system of links. They assume that every loop in this manifold can be shrunk, and ask what constraints this requirement imposes on the system of links. Then they find ways to modify the links, using those constraints, until at the end of the process they can recognize a system of links corresponding to the hypersphere. Many complicated 'moves' are needed to effect this simplification. Rourke has likened the method to solving the Rubik cube, because this also involves establishing a repertoire of complicated sequences of moves which do useful things.

The sources of technical difficulty are twofold. The first is the combinatorial complexity of the moves, and of the rules stating which one should be applied in which circumstances to achieve which tiny step in the simplification. The second is that, in order for the proof to proceed rigorously, a certain amount of technical baggage must be carried along during each move. At the end of the proof this additional superstructure can be discarded, but until the end is reached it is essential. More than one topologist has attempted to prove the Poincaré conjecture by this route, but nobody had succeeded in fitting all the pieces together and making them all work at once.

Rêgo and Rourke introduced several new ideas relating to the technical baggage, and decided that they had a proof. As far as they were concerned, these new ideas answered the question "what have you got that the others missed?". Ironically, it is one of these pieces of technical baggage that is responsible for the mistake. They impose an "ordering condition" which, roughly speaking, affects the way the various links can interpenetrate. This ordering condition makes some steps in the proof feasible, but it also carries a penalty: after each series of moves, one must check that the ordering condition continues to hold. In fact, it does not hold automatically, but Rêgo and Rourke asserted that the ordering condition could be restored by making some additional moves, which make the proof still more complicated. Unfortunately for them, this assertion seems to be incorrect in at least one case.

How do mathematicians try to avoid such mistakes in logic, and why do mistakes nonetheless occur from time to time? There are three main safeguards. One is to examine every tiny detail of the proof; the danger here is that there are so many details that one escapes attention. The second, which is safer, is to look for internal consistency, and to grasp the overall flow of the proof: if 'everything fits' then there is a fair chance that the underlying ideas are right. The third, and best, is to try out the ideas on lots of simple examples, to see if the results make sense. This is the 'experimental' approach.

The Poincaré conjecture lays several traps for the unwary. The nastiest trap has an air of Catch-22 about it. If, as one is trying to prove, the conjecture is true, then the only manifold that satisfies its hypotheses is the hypersphere. Thus the safest method, the 'experimental' one, cannot be used: the only available example is the hypersphere itself, and everything will work automatically on that!

Most aphids reproduce parthenogenetically, but some species, such as the vetch aphid *Megoura viciae*, reproduce sexually in winter (left, photograph by G. Higgins). Male vetch aphids locate their mates by detecting an attractive pheromone the females release from their hind legs. The males detect the pheromone with sense organs on the third antennal segment (right). G.W. Dawson *et al.* on page 614 of this issue show that the pheromone contains two components, nepetalactone and nepetalactol. Curiously, nepetalactol gives catmint (*Nepeta cataria* and *N. mussinii*) its characteristic smell and makes it attractive to cats. A mixture of the two compounds is as effective as the authentic pheromone for attracting males. The compounds should be useful for manipulating aphid behaviour, although commercial applications are still a long way off. ☐

Rêgo and Rourke, having developed their proof over several years, used the second method. To them, the overall flow of the ideas made good sense. They therefore concentrated on explaining this flow. In the process, they suppressed some (supposedly) routine and tedious details, a common practice in mathematical exposition, but one that always carries a certain danger.

Topologists trying to read the proof, equally reasonably, worried more about the fine details. One object of the Berkeley seminars was to fill out those routine details and check that they really were routine. Although it may be small consolation to Rêgo and Rourke, it is a tribute to the topological community that it examined the proof in enough detail to locate the error. The matemathician's natural suspicion, yet again, was vindicated.

The tale also illustrates the differences between mathematical arguments and other types of controversy: in mathematics there is, when the dust has settled, a position that is correct and one that is not. At the start of the seminar series, Rourke believed that he and Rêgo had a correct proof. But when he realized they were mistaken, he himself pointed out the error.

That is the mathematical story, but it may not be the final word. Mathematical proof is similar to pregnancy: either you have one or you don't. But a research programme, a circle of ideas aimed towards a proof, is another matter. In a research programme, logical gaps in proofs are commonplace: that is how the work progresses. If the overall idea is sound, the gaps can usually be filled. There have been cases where proofs assumed to be defective because of gaps later turned out to be fundamentally sound: the work of Kurt Heegner on the class number one problem, or Louis de Branges on the Bieberbach conjecture, are examples. There have, conversely, been gaps that cannot be bridged: previous attempts on the Poincaré conjecture and all attempts so far at the Riemann hypothesis.

Rourke and Rêgo currently see their work as a programme rather than a proof. They hope to fill in the gap, or to get round it some other way. The smart money is of course that they will fail: it always is in such circumstances. Certainly, unless they succeed in patching up their proof, they will get no credit for having solved the problem — and rightly, because they have not. The ball is now firmly in Rêgo and Rourke's court. It is up to them to prove, if they can, that their programme can be carried through. If they cannot, Poincaré's conjecture will have notched up two more victims. ☐

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AIDS virus and scrapie protein genes

SIR—Haseltine and Patarca[1] have suggested regions of sequence similarity between the 3′-end of the polymerase gene of HIV (human immunodeficiency virus, type HTLV-III)[2] and the hamster gene for PrP 27–30, a protein associated with scrapie infectivity[3,4], but Braun and Gonda have since shown that the protein sequence alignments are unconvincing[5]. Compiling scores by the Needleman–Wunsch–Sellars algorithm (program ALIGN [6]) of sequence matches between the PrP gene and available lentiviral genes, including that of HTLV-III, we show here that the alignment of the DNA sequences is also without merit.

The regions of supposed nucleotide sequence similarity between PrP and HTLV-III are largely different from those for amino-acid sequences. Numerous gaps introduced into the DNA sequences for the purpose of alignment do not correspond to gaps in the protein sequence. Haseltine and Patarca's nucleic-acid alignment has four colinear regions of homology separated by regions of poor homology and different length. As in the case of protein alignment[5], the number of gaps introduced lessens the alignment scores to the point where its significance is questionable. For example, alignment of the sequences of Fig. 1*a* of Haseltine and Patarca (PrP residues 207–631 and HTLV-III residues 4,632–5,123) with a low gap penalty of 2 (using the unitary scoring matrix of the PIR) and 100 randomizations, yields a score of 2.20 standard deviations (σ). PrP aligned with the analogous visna virus[7] and equine infectious anaemia virus (EIAV)[8] regions gives 0.87σ and -0.22σ, respectively. For comparison, alignments of the HTLV-III region with the analogous (and evolutionarily related) regions of the visna virus and EIAV genomes gives scores of 8.91 and 9.74σ at the same break penalty.

Using the program FASTN [9] to screen the GENBANK files, scores similar to, or in excess of those raised by the lentiviral-PrP alignments are frequent. For example, a section of the rat cytochrome P-450c gene[10] (residues 4,723–5,203) matches HTLV-III (residues 4,637–5,123) with a score of 3.61σ. Finally, regions other than the polymerase gene in lentiviral genomes give high ALIGN scores when compared with the PrP gene sequence. Thus, residues 5,277–5,751 of HTLV-III score 3.27σ when aligned to PrP.

Haseltine and Patarca justify many of the gaps by the existence of small local (imperfect) repeats in the HTLV-III or PrP sequences. One of these 'repeats' is broken by the omission of the hexanucleotide GTGGAA following residue 261, an error which further extends a gap in the HTLV-III sequence. Overall, the best patches of similarity occur in G–C rich parts of the amino-terminal halves of the genes.

Thus, any relationships between the amino-acid or nucleic-acid sequences of HTLV-III and PrP are probably fortuitous.

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Quantum jump

SIR—Mention of our paper[1] in a recent News and Views article[2] leads me to emphasize that our experiments merely demonstrate a jumping time as short as about one second for the electron to complete the jump from the higher to the lower level. This is considerably shorter than the approximately 30 second dwell time in the upper electronic level, and the phenomenon therefore may legitimately be described as a 'jump'. It is not significantly shorter than our earlier example of a quantum jump[3] although our later experiments have employed a different technique[4] to observe the jump, namely a new continuous type of Stern–Gerlach effect.

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Interferon-β_2 living up to its name

SIR—The B-cell differentiation factor BSF-2 (or BCDF), whose complementary DNA was recently cloned from T cells by Hirano *et al.*[1], turned out to be identical to interferon-β_2 (IFN-β_2, 26K), which we had cloned from human fibroblasts by means of its interferon activity[2-4]. Billiau[5], in pointing out this identity, questions the interferon-like function of the substance. We wish to recall the evidence which led us to classify this protein as an interferon and to consider the implications of these multiple activities.

Recombinant IFN-β_2 produced in CHO cells[3] shows the following typical IFN activities. First, it inhibits vesicular stomatitis virus, Mengo virus, and herpes simplex virus-2 in human fibroblasts and amniotic cells[3,6]. Second, it efficiently induces specific interferon-activated genes[3,4], such as (2′–5′)-oligo (A) synthetase and induces their messenger RNAs in the presence of cycloheximide, showing the action to be direct[7]. Third, it has antimitogenic effects on fibroblasts[6]. Fourth, there is species specificity [3]. And finally, IFN-β_2 displays activity on mouse–human hybrid cells containing human chromosome 21, which carries the type I interferon receptor, and the activity is inhibited by antibodies to this receptor[4,6,7]. Both the antiviral activity of IFN-β_2 and its ability to induce (2′–5′) -oligo (A) synthetase are neutralized by several polyclonal and monoclonal antibodies to IFN-β_1[3,7] (J. Vilcek and P.B. Sehgal, personal communication), but not by antibodies to IFN-α or IFN-γ, hence its designation as a β-type interferon. Structurally, IFN-β_2 has conserved half of the amino-acid residues common to all type I interferons[3]. But by its gene structure and localization, IFN-β is only distantly related to the other interferons.

The finding of BSF-2 activity in an interferon molecule active on non-lymphoid cells, is not surprising in view of the known involvement of interferons in terminal differentiation[3]. With respect to B cells, IFN-α, -β and -γ have been shown to induce chronic lymphocytic leukaemia (CLL) cells to acquire plasmacytoid characters with increased cytoplasmic immunoglobulin[8]. The growth of some CLL B cells is strongly stimulated by IFN-α[8], a finding that may be related to the plasmacytoma growth stimulation mentioned by Billiau[5]. IFN-γ has been identified as a B-cell maturation lymphokine for normal murine B cells[9] and, in the presence of interleukin-2, for human B cells[10], producing strong increases in immunoglobulin secretion. Thus, interferons have B-cell differentiation effects.

IFN-β_2, like IFN-γ, is produced by T cells, and both may exert lymphokine activity at concentrations lower than those giving antiviral effects[11]. Indeed, IFN-γ induces HLA and anti-NK cell effects 100 times more efficiently than it inhibits viruses. IFN-β_2 has a specific activity 50–100 times lower than IFN-β_1 against vesicular stomatitis virus[2,3] but its antimitogenic activity on fibroblasts is 10 times higher than IFN-β_1 (unpublished data). supporting the proposed role[12] of IFN-β_2 in fibroblast growth-control in response to tumour necrosis factor or interleukin-1. It would, therefore, be not unlikely to find differentiation affects of IFN-β_2/BSF-2 on B cells at concentrations too low to produce antiviral activity on other cells. Contrary to Billiau's comment[5], this does not mean that the inteferon activity of IFB-β_2/BSF-2 is unimportant for its function: as it is as inducible as type I interferons by double-stranded RNA and viruses in many cells[2,12], IFN-β_2 could provide an important link between the immediate tissue antiviral response and the more delayed antibody production in viral infections.

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Variations in chickens

SIR—Two recent reports in *Nature*[1,2] suggest to us a possible mechanism for the generation of antibody diversity in chickens.

During immunoglobulin light-chain gene rearrangement, recombination occurs at conserved heptamer and nonamer nucleotide sequences which flank each germline variable (*V*) and joining (*J*) gene segment. Combinational joining among multiple non-identical *V* and *J* segments is a major source of antibody diversity in the mouse and human. In contrast, the principal light-chain locus of the chicken appears to contain only one *J* and only a single functional *V* segment, and fusion of this pair is the predominant (if not the only) rearrangement event observed. Other *V*-like elements exist upstream, but those sequenced to date are pseudogenes. Nevertheless, the chicken produces diverse light chains. Diversification appears to occur through transposition of sequences from pseudo-*V* segments into the rearranged *V*/*J* locus. The mechanism of this transposition is not known, but is thought to involve gene conversion[3].

The finding[1,2,4] that transcriptionally-competent heavy-chain genes can undergo secondary rearrangements in which one *V* segment replaces another, with recombination occurring at a heptamer in the coding sequence of the first *V* segment, implies that the lymphocyte recombinase system can act on isolated heptamers contained in rearranged immunoglobulin genes. Upon examining the published sequences of the functional light chain *V* segment and three pseudo-*V* elements from the chicken, we find that each contains at least two heptamer elements within the coding region; these are located immediately upstream of the second and third complementarity-determining regions. It is unlikely that the pseudo-*V* loci undergo conventional rearrangements[3]. However, these internal heptamers, coupled with the putative heptamer-binding and endonucleolytic activities of the recombinant system, could serve as efficient sites for synapsis and strand exchange between the functional *V* segment and *V*-like pseudogenes, much as sequences flanking the mating-type loci of yeast are thought to do[5]. Resulting gene conversion events would tend to alter precisely those portions of the gene that determine antigen-specificity. Somatic diversification of the chicken light-chain genes might thus occur through a variation of the conventional heptamer-mediated recombinase pathway.

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Functions of leaf fall

SIR—To add to those already put forward[1-3], may I suggest yet two more functions for leaf fall?

Some leaves may be shed because they cease to be economical in terms of their own and/or the whole plant's energy and nutrient budget. Such shedding would have the additional advantage of allowing a better distribution of light in the plant canopy, while minimizing competition for water and other nutrients from the soil.

It also seems possible that plants may initially produce many more leaves than they require, later shedding those that are in excess of the optimum number for the normal life cycle.

The above proposals gain support from the fact that in many of the economic legume and oil-seed crops, which can experience considerable leaf fall, reproductive structures can play an important role in the overall process of building seed yields, indicating that some leaves, at least, are dispensable[4-7]. The surplus foliage can account for at least half of the total plant foliage[8]. In fact considerable defoliation of such plants can be carried out, without a significant decrease in seed yields. Thus, in all plants where reproductive structures make a considerable contribution to the final seed yields, shedding of surplus foliage could be economical in the overall metabolic context of the plant and allow better distribution of light, water and nutrients.

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Body temperature of homoiothermic animals

SIR—I agree with Calder[1] and Dunitz and Benner[2] that Paul[3] is wrong to suppose that the body temperature of many homoiothermic animals is 36 °C because that is approximately the temperature of minimum specific heat of water. Rather it may produce a paradox — homoiothermic animals live at the temperature of maximum thermal fluctuations. I also agree with Dunitz and Benner when they assert that biological water is not pure water but I disagree with them when they use the data of pure water to solve the paradox.

Indeed, water in biosystems must be thought of as adsorbed onto macromolecules, either in a localized or mobile form[4,5]. Without additional hypotheses, this model explains the Na^+/K^+ ratio[6,7], the anaesthetic effect of inert gases[8] and the anomalously high viscosity of water in the cell[4]; therefore, it must be considered as a plausible model for water in biosystems.

The observation that the specific heat of water in biosystems such as normal blood plasma and haematopoietic cells is lower (6% and 11%, respectively) than that of pure water, shows that in biological water some degrees of freedom can be excited with more difficulty than in pure water, thus sustaining the adsorbed water model.

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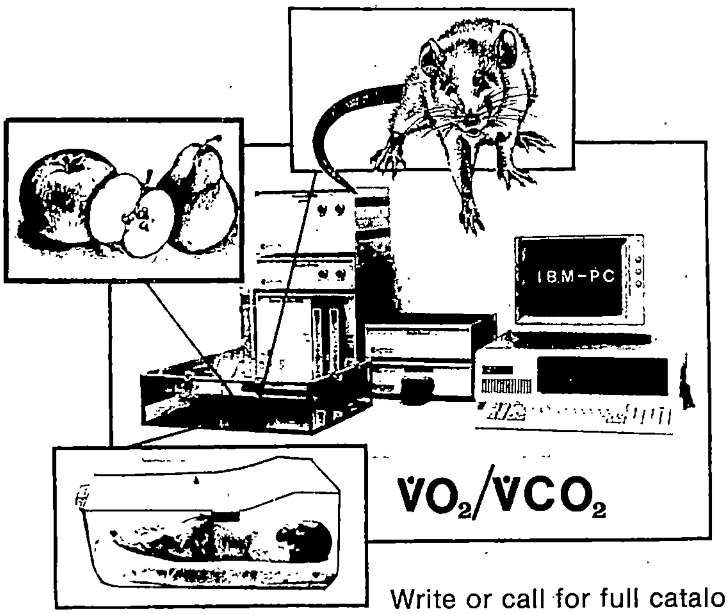

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Is seeing believing?

John C. Marshall

Blindsight: A Case Study and Implications. By L. Weiskrantz. *Clarendon: 1986. Pp.187. £19.50, $29.95.*

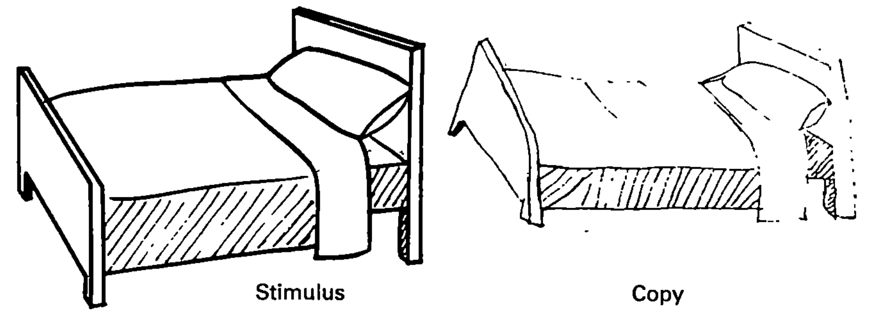
To See But Not To See: A Case Study of Visual Agnosia. By Glyn W. Humphreys and M. Jane Riddoch. *Lawrence Erlbaum: 1987. Pp.124. Hbk £12.95, $19.50; pbk £5.95, $8.95.*

IF, IN good illumination (and wearing my glasses!) my eyes alight upon a common object, I have no difficulty in recognizing that there is, for example, a chair some two metres in front of me. This is the exercise of an everyday and seemingly trivial capacity, but one which has long frustrated our best endeavours to understand perception beyond the level of early visual processing. The problem of how the brain transforms the two-dimensional mosaic of retinal stimulation into the full richness of three-dimensional objects in space thus continues to intrigue neurophysiologists, experimental psychologists and workers in artificial intelligence (who would like their 'seeing machines' to recognize not only the numbers at the bottom of cheques, but also cabbages and kings).

To this list of interested parties we should add the neurologist and the neurosurgeon, who want to understand how damage to different areas of the brain can induce blindness in different parts of the visual field, or selectively impair the perception of form, colour, stereoacuity, orientation or location in space. In healthy condition, the visual system may operate too smoothly and seamlessly for us to grasp the *modus operandi* of its component parts. Thus, as with any other complex biological function, there is the strong possibility that pathology, by rudely tearing apart and isolating the elements of an integrated mechanism, will throw light upon the structure of the normal system.

To see how counter-intuitive that light may be, we need but turn to the case-reports presented by Weiskrantz and by Humphreys and Riddoch. The man, DB, that Weiskrantz has studied was born with an arteriovenous malformation at the pole of his right occipital lobe. The medical complications (migraine, severe pain and the possibility of life-threatening haemorrhage) were such that the malformation was removed surgically when DB was 33. Of necessity, the operation involved loss of much of the calcarine cortex on the medial surface of the right hemisphere. This area contains striate cortex that (by virtue of anatomical connectivity) is functionally characterized by an orderly retinotopic mapping of the visual world onto the surface of the brain.

Unsurprisingly, then, DB was left with a visual field defect (a scotoma) that originally extended to the entire left half-field of both eyes, but later contracted to a scotoma of the lower left quadrants. In short, to perimetric testing and by all his own reports, DB was blind in these quadrants of the visual field. The puzzle is that he could reliably point to objects in this blind field, and could likewise discriminate between the horizontal or vertical orientation of a small stick exposed to the blind field. This ability surprised the patient at least as much as it did the experimenters. DB continued to insist that the tasks he had been set were ridiculous and that he was simply 'guessing' the answers to questions that a (partially) blind man could not possibly answer correctly. Seeing without believing?



Seeing without recognizing? An accurate copy of an object, but made without recognizing it, by a patient suffering from visual agnosia.

Courtesy Dr Freda Newcombe

The man, HJA, investigated by Humphreys and Riddoch suffered a stroke at the age of 60. Blockage of the posterior cerebral artery provoked bilateral damage to the occipital lobes, including primary visual (striate) cortex. HJA, like DB, had a visual field defect; in this case, he was blind in the upper two halves of both the right and the left visual field. More crucially, however, HJA could not reliably identify by sight the faces of familiar people or the common objects in his environment even when he 'saw' them in his intact visual fields, a problem exacerbated by a concomitant loss of colour vision.

Is HJA, then, only 'partially sighted', even in his supposedly good lower visual fields? No. He has normal visual acuity (glasses-corrected); he can copy very accurately a drawing of St Paul's cathedral that hangs in his living room (although it takes him six hours to do so). The accompanying picture shows the copying performance of another patient, MS, with a deficit similar to that of HJA. *We* have no difficulty in seeing the copy for what it is, although the patient himself could not recognize that the object is a bed. Has HJA 'forgotten' what familiar objects and faces look like? No. He can verbally describe their visual characteristics from memory, and he can even draw quite accurately from memory such complex objects as a screw or a biplane. (Before the war he had trained to be a pilot.) Thus in some ways, but clearly not all, HJA is in a similar position to you and I when confronted with a novel object or new face. Seeing without recognizing?

Presented with such cases, a principled scepticism is in order. Thus attempts have been made to explain away blindsight on the grounds that some residual visual function is retained within the damaged striate area. (Blindsight is a limited phenomenon, restricted to the detection of a target and relatively crude discriminations between simple stimuli.) Likewise, some of the experiments concerned have been criticized for leaving open the possibility that stray light reached intact portions of the visual field. The basic contrast between DB's retained ability to perform the tasks and his denial of 'seeing' the stimuli has led some to claim that the patient sets himself a higher 'confidence threshold' before being willing to say that he sees.

Much of Weiskrantz's book is accordingly taken up with a careful and detailed investigation of DB's visual abilities, extended over some dozen years and devoted to ruling out the obvious ways in which the interpretation of blindsight could be trivialized. To all save the direst sceptic, Weiskrantz's positive conclusions about the existence and nature of a 'second visual system', non-striate and "based on different cells of origin and different terminals, and with a different function" will seem alive and well. That the system has no (or very limited) access to conscious awareness reminds us (as does the current literature on the amnesias) that 'consciousness', anathema to the behaviourists, is not dead and will certainly not lie down.

▷

High-level visual agnosia, the deficit from which HJA suffers, has likewise been dismissed as an impossibility. Many neurologists have tried to explain away the performance of such patients by claiming that they suffered from a combination of low-level sensory problems aggravated by a generalized dementia. Once again, Humphreys and Riddoch's meticulous testing shows that such an explanation will not suffice. Apart from his problems with the visual recognition of objects, faces and written words, and with finding his way around his home town, HJA remains a highly capable and intelligent human being. He has excellent insight into his deficiency, and can describe his difficulties with remarkable cogency, although no words will do justice to the subjective feel of how his visual world has changed. Moreover, he has been able to devise sensible strategies for coping with the perceptual, intellectual and emotional consequences of his stroke.

Although these monographs provide appropriate literature-reviews and outline whatever background anatomical, physiological and psychological evidence is relevant to their theses, in both instances the primary data come from the study of a single individual. This provides striking confirmation of the methodological shift that cognitive neuropsychology has recently undergone. Group studies, in which a small range of observations is collected from a large sample of patients who are only superficially similar, either in terms of neuropathology or behavioural deficit, have proved an inadequate foundation on which to build models of human cognition. It is now widely recognized that single case-studies provide a more compelling, less ambiguous, database for inference to underlying mental representations and processes. Such investigations can furnish enough observations on the one patient to enable one to home in on the precise information-processing mechanisms involved in the pattern of impaired and preserved performance. The research strategy is well illustrated in the sequence of experiments whereby Humphreys and Riddoch isolated HJA's primary deficit: a disorder in " 'binding together' the local parts of objects simultaneously at different spatial locations".

Of course, the deficits of one patient cannot reveal the overall structure of the visual system. But from a series of cases, each with a different selective disorder, we may reasonably hope to discover the functional architecture of visual cognition. It is in this sense that *Blindsight* and *To See But Not To See* are important steps towards the eventual maturity of cognitive neuropsychology as a natural science. □

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The comparative advantages

Clive R. Bagshaw

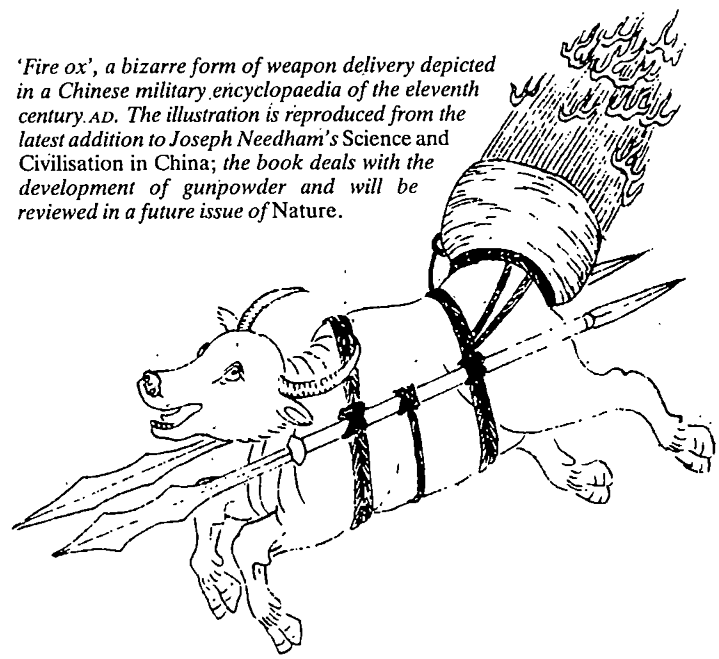
Calcium in Muscle Activation: A Comparative Approach. By Johann Caspar Rüegg. *Springer-Verlag: 1986. Pp.300. DM 198.*

As more problems of biology approach a molecular level, the chemical and physical sciences make an ever-increasing contribution to the advances in our understanding. Comparative physiology might seem a backwater, followed by those whose originality limits them to choose a new organism rather than a new experiment upon which to work. Rüegg sets out to dispel this viewpoint, quoting in the Introduction his predecessor Krogh, who advised experimentalists to search for the most specialized organisms or tissues in order to explore mechanism.

Throughout the book, many examples are given where new experiments were made possible by the choice of tissue rather than an advance in technology *per se*. Take Ca^{2+} itself — the central theme of this work. Muscle physiologists were aware of the importance of this ion as a messenger long before its acceptance as a trigger in a host of other cellular functions. With a head start of nearly a century, the molecular bases of activation mechanisms are better understood in muscle (but by no means solved) than in any other system. Indeed, calmodulin was known as troponin-C-like protein just a decade ago, before its explosive rise to fame in metabolism as a whole.

A second strength of the comparative physiological approach is as an aid to understanding the generation of diversity through evolution. Already the euphoria of some protein engineers has been dampened by the realization that nature has had more than 10^9 years to experiment with amino-acid substitutions. Its successes are here today, but are in danger of being forgotten as classical zoology and botany are squeezed out of the syllabus. Muscle from different organisms shows over a 10,000-fold range in shortening speeds. This is accomplished by diversity in the rate and mode of stimulation, the diffusion distance of the Ca^{2+} message, and the properties of the Ca^{2+}-receptor proteins, Ca^{2+} pumps and contractile machinery. Input is therefore required from plant and animal anatomy, physiology and biochemistry to gain a full understanding. Yes, plants do have muscles of a sort and knowledge of their anatomy has provided an elegant way to demonstrate the velocity of shortening is, in part, controlled by the particular myosin isoenzymes present in a muscle. The giant algal cell *Nitella* contains strands of actin running along its length which provide an *in vitro* substrate along which latex beads, covered with myosin, crawl with a velocity dependent on the muscle source. But much of the diversity of muscles is a reflection of their microanatomy which would be missed by the study of isolated proteins alone.

There are few people of Rüegg's experience to document these comparative aspects of activation of muscle contraction so thoroughly, and who could blend the disciplines involved so effectively and do justice to both the history of the subject and the latest discoveries. The individual



'Fire ox', a bizarre form of weapon delivery depicted in a Chinese military encyclopaedia of the eleventh century AD. *The illustration is reproduced from the latest addition to Joseph Needham's* Science and Civilisation in China; *the book deals with the development of gunpowder and will be reviewed in a future issue of* Nature.

chapters are more or less self-contained, and cover excitation–contraction coupling mechanisms in a variety of vertebrate and invertebrate muscle types. There is some repetition, but this is no bad thing as most readers are likely to refer to a section at a time. The writing is lucid and most of the figures have been redrawn from the literature in a clear, consistent format. Controversies are generally dealt with by presenting both sides of the argument, without forcing premature conclusions.

Nevertheless, the various topics are held together with the author's own views, which the reader may or may not share. Thus, in a comparison of actin- and myosin-linked regulation, Rüegg considers that the former is more efficient because there is "a built-in guarantee that crossbridges do not hydrolyse ATP unless they are attached to actin", yet it is known that skeletal myosin alone hydrolyses ATP *in vitro* at a suspiciously high rate compared with a relaxed muscle. Some additional way of modulating the myosin ATPase may therefore be implicated. □

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All about leeches

Ronald W. Davies

Leech Biology and Behaviour. By Roy T. Sawyer. *Oxford University Press: 1986. Pp. 1,065 in three volumes. Vol. I £47.50, $85; Vol. II £42.50, $85; Vol. III £25, $50.*

Perhaps the question that almost all invertebrate zoologists would ask about books of the type written by Sawyer is "How do they compare to Libbie Hyman's volumes on the invertebrates?". The short answer is in many respects very well, but in other respects poorly.

The three volumes — *Anatomy, Physiology, and Behaviour*; *Feeding Biology, Ecology, and Systematics*; and *Bibliography* — cover almost all aspects of the subject matter implied by their titles, but the emphasis is upon the use of leeches in modern medicine and particularly the numerous chemicals of pharmacological use produced by leeches. Each topic is covered extensively and as a source of information the books are excellent. If, however, the reader is looking for an holistic approach fitting leeches into a more general framework, there are disappointments. This particularly applies to the ecological section, which has little interpretative content and fails to establish substantive links with mainline ecology.

Thus, like Hyman, if information is required Sawyer will generally provide it. But, unlike Hyman, there is no balanced analysis of possible phylogenetic development. Only an enthusiastic leech researcher would seriously contemplate reading these books from start to finish, but by beginning with a very personal and idiosyncratic account of the phylogenetic affinities of the leeches Sawyer will probably deter all but the most dedicated. My greatest fear is that these introductory chapters may well not only put off the casual reader but will potentially misinform or bias the unwary. For example, Sawyer suggests that certain traits reflect a monophyletic link between the Clitellata and the Uniramea, with the Polychaeta having a separate and distinct origin. Alternative views are largely ignored or barely mentioned. The dogmatic adherence to this phylogenetic perspective pervades almost every chapter and detracts from an otherwise excellent work of scholarship. Similarly, the inclusion of the Acanthobdellida and Branchiobdella within the leeches (Hirudinea) deserves more discussion, and alternative phylogenetic pathways should have at least been mentioned.

The sections on systematics, evolution and zoogeography will be extremely useful to leech researchers throughout the world, although it is a pity that Sawyer does not fully explain the taxonomic changes he has instituted. Most leech researchers are well acquainted with the publications of Soos and Richardson on leech genera of the world (published in *Acta Zoologica Academiae Scientiarum Hungaricae*). However, many of the genera, especially in the Hirudinidae, so carefully defined by these workers have been eliminated largely without explanation. Inclusion of this information would have undoubtedly increased the value of Sawyer's books.

The separation of the chapter on behaviour in Vol. I from those on feeding biology and ecology in Vol. II is difficult to understand, as is the separation into different volumes of phylogeny and systematics. With a common index for Vols I and II and the bibliography in Vol. III, most readers will be required to juggle two if not three volumes coincidentally. Undoubtedly space was saved as a result, but at the cost of seriously inconveniencing the user.

The books are well written and illustrated, and despite (or perhaps because of) Sawyer's personal biases on phylogeny I found them enjoyable and stimulating. The author must be congratulated on a monumental achievement, which will be invaluable to dedicated leech researchers and a must for their laboratories and every university library. □

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Malevolent moulds on record

Maurice O. Moss

Fusarium Species: Their Biology and Toxicology. By Abraham Z. Joffe. *Wiley: 1986. Pp.588. $74.95, £71.75.*

It was just six years ago that a speech made in West Berlin by US Secretary of State Alexander Haig threw the mould genus *Fusarium* into the spotlight of the news media. The speech linked the phenomenon of 'yellow rain' in South-East Asia with the production and use of the toxic mould metabolites known as trichothecenes as agents of chemical warfare. Although 'yellow rain' is probably the pollen-laden faeces of bees, and the truth about the use of chemical weapons in South-East Asia is distorted by the murky clouds of superpower politics, the importance of *Fusarium* in natural cases of poisoning of man and domesticated animals is well recognized.

Severe outbreaks of illness associated with the consumption of cereals contaminated with species of *Fusarium* and other moulds occurred in parts of the Soviet Union during the first half of this century. This illness, known as alimentary toxic aleukia (ATA), is associated with a complex pattern of debilitating disorders which starts with inflammation of the mouth and throat and is followed by damage to the mucosal membrane systems of the intestine and associated vomiting and diarrhoea. With increased consumption of toxic cereals, damage to the bone marrow occurs followed by a complex anaemia. The trichothecenes are now considered to be responsible for ATA. Like several other mycotoxins they are immunosuppressive, so the body's defences against bacterial and viral infections are reduced; indeed, many deaths during outbreaks of ATA were put down to pulmonary and other infections.

During a major Russian outbreak of ATA in the 1940s, Abraham Joffe was Director of the Mycology Laboratory of the Institute of Epidemiology and Microbiology in Orenburg. He was closely involved in investigations of the aetiology of the disease and continued to specialize in the study of toxigenic fusaria as head of the Laboratory of Mycology and Mycotoxicology at the Hebrew University of Jerusalem. *Fusarium* is only one of a number of mould genera which are known to produce toxic metabolites (zearalenone, butenolide and moniliformin, for example, as well as the trichothecenes) and there is now a considerable body of literature on the mycotoxigenic fungi, mycotoxins and mycotoxicoses. Professor Joffe has made continuous and notable contributions to this literature, and the book under review represents the accumulation of his experience with *Fusarium.*

The book begins with a brief historical background account, which is followed by chapters on the toxigenic fusaria and their principal toxins; included here are tables of yields of toxins produced in the laboratory, concentrations found during instances of natural occurrence, and values, taken from the literature, of their toxicity in terms of LD_{50}. Next come discussions of antibacterial, antiprotozoal and insecticidal activity (but not of antifungal activity, despite the existence of several reports on the use of yeasts in the bioassay of trichothecenes) and of the phytotoxicity of *Fusarium* species. The latter chapter is dominated by the author's own studies on correlation between the rabbit skin test and phytotoxicity — one hopes that such tests will soon be made obsolete by developments in immunological assays, biological assays using microorganisms and cell cultures, and modern physicochemical analytical methods.

The remaining chapters are devoted to human fusariotoxicoses, the role of *Fusarium* in human mycoses, fusariotoxicoses of laboratory and domestic animals, and the difficult issues of the taxonomy and identification of toxigenic isolates (keys and detailed descriptions and synonymies are provided). Professor Joffe finishes where I began — with a comment on the question of 'yellow rain'.

The contents are essentially a compilation of primary data, and this is not an easy book to read. It gives the reader the feeling that he or she is looking over the author's shoulder directly into his laboratory records. Compounding the problem is the large number of references interspersed throughout the text. The resulting bibliography fills 86 pages and spans the period from 1890 to 1984.

Despite the difficulty of assimilating all of the information presented in this book, no one will be left in any doubt about the toxigenic nature of many isolates of *Fusarium*. A bonus for those interested in biological variability is that it is unusual to find comparative data on such a large collection of isolates, and the book provides useful insight into the range of toxigenic capability amongst isolates of the same species. ☐

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How to play with particles

Robert J. Hollebeek

Introduction to Experimental Particle Physics. By Richard C. Fernow. *Cambridge University Press: 1986. Pp. 421. £32.50, $44.50.*

Statistics for Nuclear and Particle Physicists. By Louis Lyons. *Cambridge University Press: 1986. Pp.226. £25, $44.50.*

Detectors for Particle Radiation. By Konrad Kleinknecht. *Cambridge University Press: 1986. Pp.206. £25, $44.50.*

EXPERIMENTAL elementary particle physics has changed so rapidly during the past decade that few of its experts have attempted to describe the current state of research in anything but conference proceedings and contributions to technical journals. The problem is that having spent years attempting to write a comprehensive review of the subject, an author may find himself in possession of an excellent but out-dated volume. For the student, however, this situation makes it quite difficult to become an expert except by working directly on a high-energy physics experiment and reading extensively in current journals. Even this is sometimes not enough, since many experiments are now done by several hundred physicists and take many years to complete. As a result, a student may be quite knowledgeable in a specific area, and yet know little about many of the other techniques being used on his own experiment. It is a brave author indeed who embarks on publishing a text in such a field, and the rest of us should be suitably thankful that some still do.

Richard Fernow's *Introduction to Experimental Particle Physics* is an excellent step towards bridging the gap between the enormous amount of primary literature and the beginning graduate student or interested scientist. The emphasis here is not on theories but on techniques. If you wish to learn about quarks, unified field theories, strings, supersymmetry and weak gauge theories, you should look elsewhere; but if you want to learn about the many techniques used to design a detector capable of probing inside the smallest distances known to man, this is a good place to start.

The introductory chapters cover the various interactions of charged and neutral hadrons and leptons in matter in sufficient detail for a graduate-level course. The author proceeds from this background to discuss the types of detectors which make use of these physical processes, the electronics systems used to read them, and the machines and beams used to do the physics. In many cases, comparisons are made between different techniques and suggestions given to the experimenter on how to optimize a detector for a particular application. There are a large number of references, many of which cite major review articles or important journal papers, and each chapter concludes with a small number of exercises.

Also of interest to experimental particle physicists, as well as anyone else who has to *use* statistical techniques, is Louis Lyons's *Statistics for Nuclear and Particle Physicists.* This is probably not the first book on statistics which a student or researcher should read, because many results from statistical analysis are stated or provided in a non-rigorous way. It is instead, as the author states, the result of "a course given by a non-statistician to non-statisticians". While the title states that the book is intended "for nuclear and particle physicists", this is probably too restrictive; many examples are drawn from particle physics, but they are simple enough to be understood by the non-expert.

The book's true value is that it contains the accumulated experience and insight of someone who has learned to use statistics to solve problems encountered by scientists in general. Many simple examples are given in which the author emphasizes the connections between various statistical techniques and the most frequent errors made in applying them. This, then, is an ideal book for the person who knows about statistical techniques but wants to learn how and why to apply them. In considering the origin of the well-known N-1 term in the sample variance, for example, Lyons points out the advantages and disadvantages of using several other choices of the form $N + k$ (one instance is that using $N + 1$ yields a biased estimator, which has a smaller variance about the true variance). When correlated variables are introduced, Lyons starts with the simple example of Abram and Lot at the cross-roads. In Lyons's words, "The reader interested in further details should consult Genesis 13:9". A challenging set of problems accompanies each chapter, many of which expand on concepts discussed in the text.

Detectors for Particle Radiation by Konrad Kleinknecht is the most detailed of the books under review, and consequently will become dated most rapidly. Several of the areas covered are already in need of changes and additions; for example, semiconductor detectors are described but no mention is made of advances in the past few years in CCD and silicon strip detectors, and the account both of RICH counters and compensation in hadron calorimeters needs to be updated. The discussion of the physical processes which are used in different types of detectors is briefer than Fernow's, and probably less useful for graduate students, but researchers using wire chambers or scintillators will find here an outstanding review of their speciality. ☐

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Seasonal temperatures in Britain during the past 22,000 years, reconstructed using beetle remains

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Seasonal and mean annual temperatures can be reconstructed from radiocarbon-dated beetle remains to yield a synthesis of the palaeoclimatic history of Britain over the last 22,000 years. The reconstructions agree well with independent evidence from periglacial features and provide a detailed picture of the glacial to interglacial transition 14,500–9,000 years ago.

IN this paper we present a detailed reconstruction of temperature changes for both summer and winter in the British Isles, based upon radiocarbon-dated occurrences of fossil *Coleoptera* (beetles) covering the period between the last glacial maximum and the present day. Britain is a particularly suitable area for such study since it lies at the south-west edge of the former European ice-sheets, and adjacent to the region of the North Atlantic which was traversed by migrations of the front between polar and sub-polar water masses[1]. There is also a 30-year history of studies of fossil *Coleoptera* and their climatic interpretation, which provides data which are unmatched elsewhere in the world.

Principles of palaeoclimatic reconstruction

The palaeoclimatic reconstructions described below were made by a new technique, the mutual climatic range method, which is derived from early work on pollen by Iversen[2] and Grichuk[3]. The method has been fully described elsewhere[4] but the principle is extremely simple. The basic assumption is that, if the present day climatic tolerance range of a beetle species is known, then fossil occurrences of that species imply a palaeoclimate which was within the same tolerance range. If several species occur together as fossils, then the palaeoclimate of the time when they were living must lie within the mutual intersection of their tolerance ranges. In general the intersection (or Mutual Climatic Range) will be smaller and the palaeoclimate deduced more precisely, the larger the number of co-existing species. Figure 1*a* shows two tolerance ranges and their mutual climatic range (MCR).

Coleoptera are especially suited to this technique because they are a varied group in which many species show fairly well defined tolerance ranges, they are well preserved as fossils, and almost all Pleistocene occurrences can be identified as belonging to living species. It has been shown[5] that on a hemispheric scale the distribution of individual species reflects the thermal climate, especially the warmth of the summer and the degree of continentality or temperature contrast between summer and winter, and that the species compositions of fossil beetle assemblages respond rapidly to climatic change. This last point has been confirmed by Hengeveld[6] who has shown that the relative abundances of species making up the present-day fauna of the Netherlands has changed in response to climate on a decadal timescale since 1890.

For this study, we have defined the climatic tolerance ranges of 350 beetle species which occur as Pleistocene fossils. Only carnivorous species from the families Carabidae, Dytiscidae, Gyrinidae, Haliplidae and Hydrophilidae and dung-beetles (Scarabaeidae) were included. We have avoided using phytophagous beetles, whose distributions might reflect that of their host plants. A map of each species' present distribution was compiled from information in the entomological literature and museum specimens. Each map was then transformed into a diagram of the species' distribution in terms of climatic variables, by superimposing the geographical range onto a base map of 495 climate stations scattered across the Palaearctic region from Greenland to Japan. Principal components analysis of the mean monthly temperatures from these stations shows that over 96% of the variance in thermal climate of the Palaearctic is described by two groups of variables (principal components) which can be interpreted as (1) the warmth of the summer and (2) the temperature range between warmest and coldest months. As the mean temperature of the warmest month (TMAX) is almost perfectly correlated with the first component, and the temperature range (TRANGE) is similarly correlated with the second component, these two variables were used directly to express the warmth of the climate and its degree of continentality.

The 495 stations were plotted on a diagram of TMAX against TRANGE. For each species, the stations which lie within its geographical range were distinguished, from which can be determined the species climatic range (SCR). The validity of this procedure was demonstrated when disjunct geographical distributions became, on replotting, coherent climatic ranges.

To reconstruct the MCR of an assemblage of coexisting species, the SCRs of all members of the assemblage are superimposed, as in Fig. 1*a*. From the extent of the MCR on such a diagram, we may read off the ranges of TMAX and TRANGE within which the palaeoclimate should fall. The range of mean temperatures of the coldest month (TMIN) may also be read from the diagram. Of course, we cannot be sure exactly what value the palaeoclimate had, only that it lay somewhere within the MCR.

A great advantage of the MCR is that it is possible to test its accuracy by reconstructing the climate from living assemblages of *Coleoptera* and checking the results against nearby climate stations. We have applied the method to lists of the species found in 15 restricted localities (such as nature reserves and small islands) in Iceland, Europe and Siberia, plus one site in Labrador. In Fig. 1*b* the reconstructed ranges of possible values for TMAX and TMIN are compared with the actual climates of each locality. There is excellent agreement. Overall, the MCR method provides an accurate indication of the range of values within which the climate of an assemblage should fall.

We now turn our attention to the problem of estimating the 'most probable' value of the palaeoclimate within the MCR. As a first approximation we may take the median values of the possible ranges for TMAX and TMIN. However, Fig. 1*b* shows that these tend to provide a systematic deviation from the 45° line with the reconstructed median lying above the true value of TMAX in all but the most temperate climates. With TMIN, the median overestimates the true value in colder climates but underestimates it for localities with mild winters. These systematic deviations can be corrected using regression equations of the present-day temperatures at various localities against the median reconstructed temperatures based on the *Coleoptera* from the same localities. To derive these regressions we used two independent sets of data comprising, firstly, the fifteen localities shown in Fig. 1*b* and, secondly, faunal lists from 31 geographical provinces of Scandinavia and Denmark reported by Hansen *et al.*[7]. The latter must be used with caution because the lists comprise species collected over wide areas with, in some cases, internal variations in climate, and may include species whose climatic ranges do not overlap. Nevertheless, when separate regressions for TMAX and TMIN were derived from each data set and tested on the other, satisfactory results were obtained, so the two data sets were combined to produce the correction equations,

$$\text{TMAX(corrected)} = 1.066\,\text{TMAX(median)} + 0.0142\,\text{NSPEC} - 2.96 \quad (1)$$

$$(r = 0.94;\ s = 0.83\,°\text{C})$$

$$\text{TMIN(corrected)} = 1.416\,\text{TMIN(median)} + 1.904 \quad (2)$$

$$(r = 0.94;\ s = 2.42\,°\text{C})$$

where temperatures are in °C and NSPEC is the number of species used in the reconstruction. Full details of this calibration procedure will be published elsewhere. 'Corrected' values obtained using equations (1) and (2) provide unbiased estimates of the most probable palaeoclimate within the MCR, with a precision of ~±2 °C for TMAX and ~±5 °C for TMIN.

Results for British Isles, 22,000 yr BP to now

The MCR method has been applied to 57 coleopteran faunas from 26 sites in England, Ireland and Southern Scotland. Site locations are shown in Fig. 2*a* and details are given in Table 1. Forty of the faunas are directly associated with a radiocarbon date on material from the same stratum. At only two sites, Glanllynnau[9] and St Bees[10], was there a sufficiently long and detailed stratigraphic section to allow dating by interpolation between radiocarbon-dated horizons. Between them, these two sites provided 6 directly dated faunas and 17 faunas dated by interpolation between 14,500 and 11,500 yr BP.

The results are shown in Fig. 2*b* and 2*c*. Figure 2*b* presents values of TMAX and TMIN in the form of vertical bars indicating the MCR of each fauna. The position of each bar on the time axis of the diagram is given by its radiocarbon or interpolated date. Dating uncertainties are not shown. One-sigma errors vary from ±45 to ±325 yr, averaging ±120 yr ($s = 62$).

Figure 2*b* shows several periods of very rapid climatic change, especially the dramatic warmings at ~13,000 yr BP and ~10,000 yr BP and a slower cooling from ~12,500 to 10,500 yr BP. These changes occur progressively over a series of points on the graph and their timing and rapidity are therefore well established. However, there is also weaker evidence for minor excursions of the climate away from the major trends. The question of whether these minor excursions are real features or mere artefacts of the uncertainty in the data depends critically upon the accuracy and precision of individual radiocarbon dates.

Figure 2*c* was prepared as an attempt to overcome some of the dating uncertainties surrounding individual points on Fig. 2*b*, by presenting time-averaged temperature estimates calculated at 50-year intervals. For each 50-year point, all the temperature estimates whose dates overlapped the point at the 3σ level were identified. The weighted mean of these temperatures was then calculated, using weightings determined as follows. The separation of each dated temperature estimate from the time point under consideration was expressed as a multiple, X, of the 1σ standard error of the date. The weighting factor for that temperature estimate was calculated as the ordinate of the normal curve at distance $X\sigma$, from the mean. Thus, each individual data point in Fig. 2*b* has been 'spread' over a time range determined by the precision of its date and combined with other points similarly 'spread'. This procedure is not totally satisfactory in that its effects depend somewhat upon the spacing of dates in time as well as their uncertainties, but it is an objective way of producing a single line through the scatter of data. The influence of single anomalous points is reduced and the timing of major changes reflects the uncertainties of all of the individual dates.

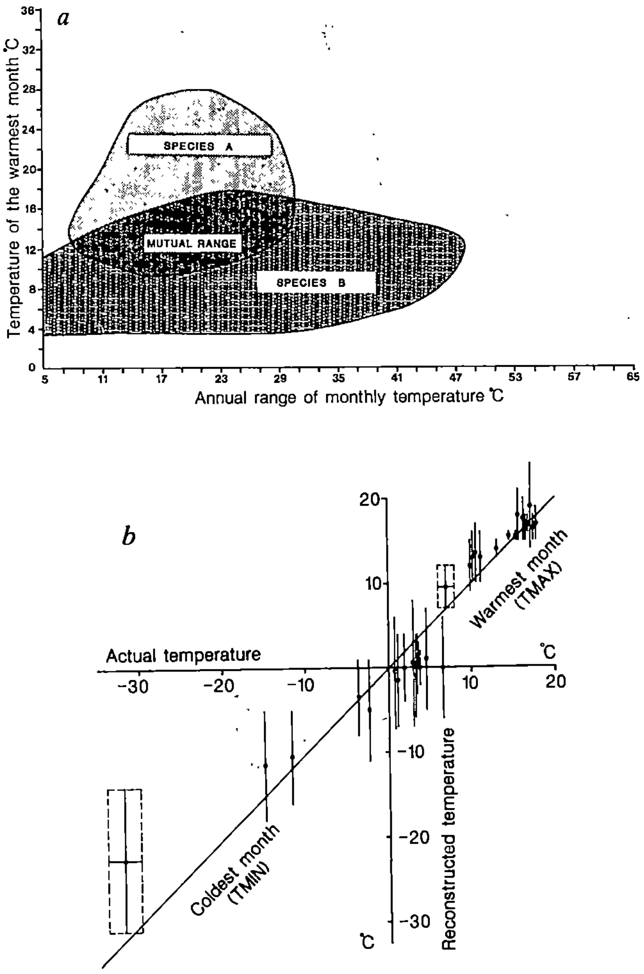

Fig. 1 *a*, Tolerance ranges and mutual climatic range (MCR) of two species of coleoptera. The tolerance ranges were determined by the method described in the text. *b*, Test of the MCR method on assemblages of species found living today at fifteen localities in Europe, Iceland and Siberia. The reconstructed temperatures of the warmest and coldest months of the year (TMAX and TMIN) are shown on the vertical axis. Points representing TMIN lie below and to the left of the upper boxed point. Vertical bars are not conventional error bars. They indicate the MCR, that is the predicted range of temperature within which all of the species at each locality could co-exist. Dots show the median value of the MCR at each locality. The horizontal axis shows the actual mean temperatures at climate stations close to each locality. Boxed points represent the coldest locality studied, Chaun Bay in north-west Siberia, for which actual temperature data were interpolated from a climatological atlas. The MCR method gives an accurate prediction of the climate for all points whose vertical bar overlaps the 45° diagonal line.

The bold lines shown in Fig. 2*c* represent the best estimates of palaeotemperature obtained by applying equations (1) and (2) to the median values of the MCR. The shaded lines show the upper and lower bounds of the MCR itself. Thus, the most

Table 1 Locations and dates of Coleopteran faunas

Site*	Number on Fig. 2	UK National Grid Reference	Date§	Lab. No.	NSPEC‖	Publication reference
Lea Valley, N. London	1	TQ357936	21,580±480	BIRM-238	5	33
Barnwell Station, Cambridge	2	TL462573	19,500±650	Q-590	12	34
Dimlington, E. Yorks	3	TA391271	18,370±334	I-3372	4	35
Glanllynnau, Gwynnedd	4	SH455373	14,470±300	BIRM-108 BIRM-212	6	9
Glanllynnau, Gwynnedd	4	SH455373	14,110±380	Interpolated	6	9
Glanllynnau, Gwynned	4	SH455373	13,840±380	Interpolated	3	9
Glanllynnau, Gwynnedd	4	SH455373	13,570±380	Interpolated	2	9
Stafford, Staffs.	5	SJ927233	13,490±375	BIRM-150	5	36
Colnbrook, W. London	6	TQ026776	13,405±170	Q-2021	6	37
Glanllynnau, Gwynnedd	4	SH455373	13,300±380	Interpolated	2	9
Glanllynnau, Gwnnedd	4	SH455373	13,165±380	Interpolated	4	9
Glanllynnau, Gwynnedd	4	SH455373	13,030±380	Interpolated	6	9
Roberthill, Dumfreisshire	7	NY110797	12,940±250	Q-643	7	38
Glanllynnau, Gwynnedd	4	SH455373	12,925±380	Interpolated	10	9
Glanllynnau, Gwynnedd	4	SH455373	12,860±380	Interpolated	8	9
Glanllynnau, Gwynnedd	4	SH455373	12,725±380	Interpolated	10	9
Glanllynnau, Gwynnedd	4	SH455373	12,655±380	Interpolated	21	9
Glanllynnedd, Gwynnedd	4	SH455373	12,590±380	Interpolated	50	9
St. Bees, Cumbria	8	NX965114	12,560±170	BIRM-378	43	10
Glanllynnau, Gwynnedd	4	SH455373	12,556±230	BIRM-276	59	9
St. Bees, Cumbria	8	NX965114	12,405±210	Interpolated	25	10
St. Bees, Cumbria	8	NX965114	12,255±210	Interpolated	28	10
West Bromwich, W. Midlands	9	c.SP020940‡	12,165±160	IGS-C14/11 St. 3059	4	39
Shortalstown, Co. Wexford	10	T303114†	12,165±180	I-4963	20	40
St. Bees, Cumbria	8	NX965114	12,100±210	Interpolated	21	10
St. Bees, Cumbria	8	NX965114	11,945±210	Interpolated	21	10
St. Bees, Cumbria	8	NX965114	11,790±210	Interpolated	18	10
Lea Marston, Warwicks.	11	SP210940	11,700±200	BIRM-208	21	41
Bigholm Burn, Dumfriesshire	12	NY316812	11,700±180	Q-694	25	38
St. Bees, Cumbria	8	NX965114	11,635±210	Interpolated	14	10
St. Bees, Cumbria	8	NX965114	11,500±120	BIRM-647	24	10
Wilden, Worcs.	13	SO824730	11,420±480	BIRM-1020	4	42
St. Bees, Cumbria	8	NX965114	11,300±220	BIRM-648	19	10
Northmoor, Oxon.	14	SP419041	11,250±100	BIRM-105	9	43
West Drayton, W. London	15	TQ053792	11,230±120	Q-2030	6	37
Redkirk Point, Dumfriesshire	16	NY301652	11,205±177	BIRM-41	28	38
St. Bees, Cumbria	8	NX965114	11,180±120	BIRM-649	31	10
Redkirk Point, Dumfriesshire	16	NY301652	10,898±127	BIRM-40	24	38
Farmoor, Oxon.	17	SP452059	10,600±250	BIRM-590	22	43
Brimfield, Herefordshire	18	SO389681	10,625±282	BIRM-404	21	44
Drumurcher, Co. Monaghan	19	H521172†	10,515±195	BIRM-239	20	45
St. Bees, Cumbria	8	NX965114	10,430±165	BIRM-1144	8	10
Croydon, Surrey	20	TQ297656	10,130±120	BIRM-101	5	46
West Bromwich, W. Midlands	9	c.SP020940‡	9,970±100	IGS-C14/12 St. 3060	7	39
Wilden, Worcs.	13	SO824730	9,930±220	BIRM-905	10	42
Wilden, Worcs.	13	SO824730	9,860±240	BIRM-907	2	42
Brighouse Bay, Kirkudbrightshire	21	NX634452	9,640±180	Q-398	15	38
West Bromwich, W. Midlands	9	c.SP020940‡	9,540±100	IGS C14/82 St. 3693	3	39
Lea Marston, Warwickshire	11	SP210940	9,450±200	BIRM-215 BIRM-310,311,312 BIRM-329	38	47
West Bromwich, W. Midlands	9	c.SP020940‡	9,080±455	IGS-C14/83 St. 3688	5	39
Alcester, Warwickshire	22	SP099577	8,480±140	BIRM-709	4	48
Alcester, Warwickshire	22	SP099577	8,320±100	BIRM-860	5	48
Shustoke, Warwickshire	23	SP227908	4,830±100	NPL-39	19	49
Wilsford, Normanton Gorse, Wilts.	24	c.SU1280‡	3,330±90	NPL-74	25	50
Porthmear, Cornwall	25	SW848715	3,024±126	BIRM-197	4	51
Fisherwick, Staffs.	26	c.SK175098‡	2,130±100	BIRM-614	19	52
Shustoke, Warwickshire	23	SP227908	410±90	NPL-62	25	49

* Sites are given in order of date and can be identified on Fig. 2*a* by number and on Fig. 2*b* by counting bars from the left.
† Irish Republic Grid Reference on 1:68360 maps.
‡ Approximate Grid Reference (not given in relevant publication).
§ Radiocarbon Years BP with 2σ counting error.
‖ Number of species in MCR method.

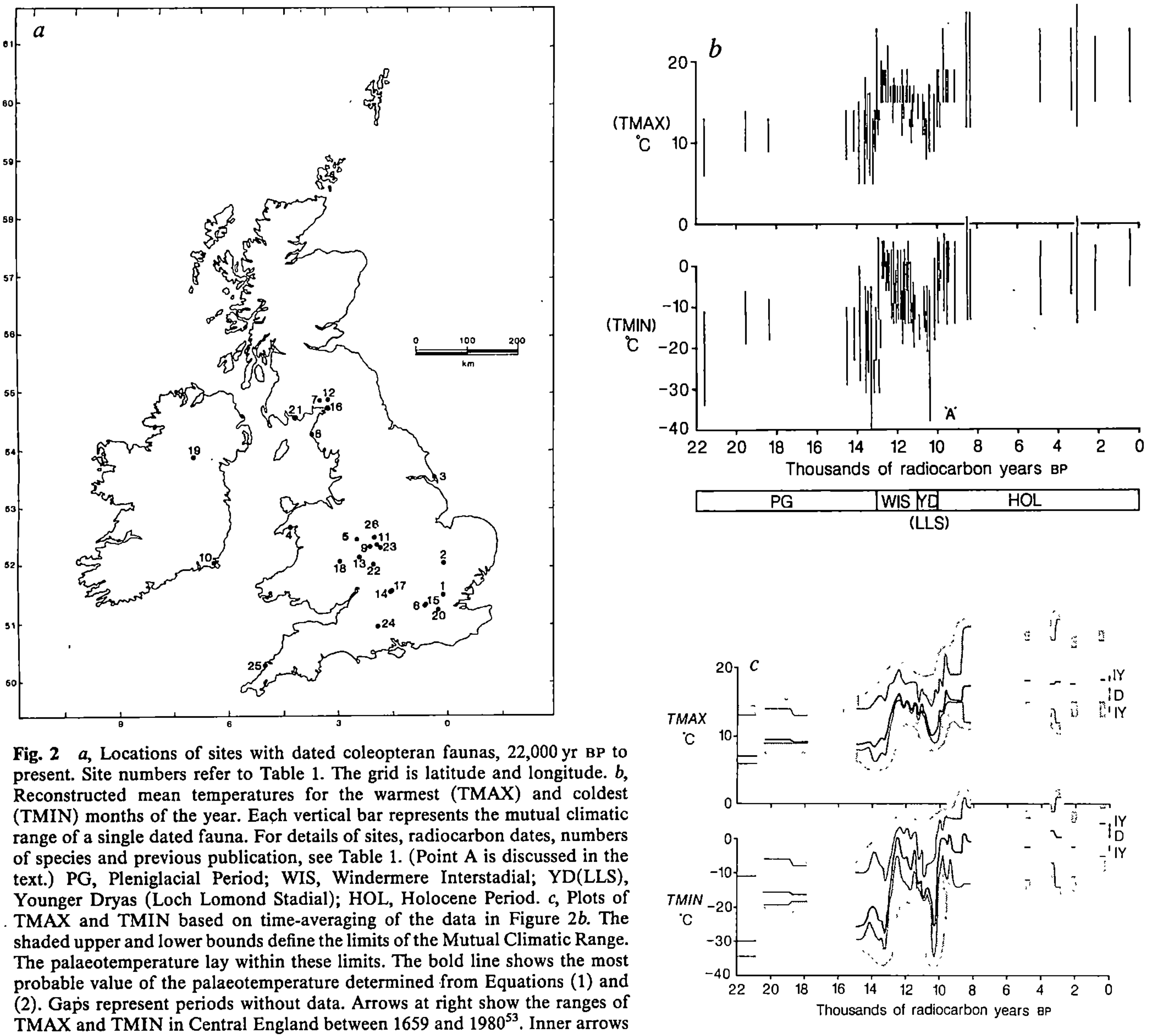

Fig. 2 *a*, Locations of sites with dated coleopteran faunas, 22,000 yr BP to present. Site numbers refer to Table 1. The grid is latitude and longitude. *b*, Reconstructed mean temperatures for the warmest (TMAX) and coldest (TMIN) months of the year. Each vertical bar represents the mutual climatic range of a single dated fauna. For details of sites, radiocarbon dates, numbers of species and previous publication, see Table 1. (Point A is discussed in the text.) PG, Pleniglacial Period; WIS, Windermere Interstadial; YD(LLS), Younger Dryas (Loch Lomond Stadial); HOL, Holocene Period. *c*, Plots of TMAX and TMIN based on time-averaging of the data in Figure 2*b*. The shaded upper and lower bounds define the limits of the Mutual Climatic Range. The palaeotemperature lay within these limits. The bold line shows the most probable value of the palaeotemperature determined from Equations (1) and (2). Gaps represent periods without data. Arrows at right show the ranges of TMAX and TMIN in Central England between 1659 and 1980[53]. Inner arrows (D): decadal means. Outer arrows (IY): individual years.

probable value of temperature at any time is given by the bold line, and it is extremely unlikely that the actual temperature fell more than one or two °C outside the range shown by the shaded lines.

Rates and timing of climatic changes

The record of summer temperatures and climatic change shown in Fig. 2 is in substantial agreement with earlier interpretations based upon *Coleoptera*[5,11,12]. However, the method described here has enabled the reconstruction of a detailed series of winter and mean annual temperatures. Figure 2*c* shows variations in temperature on two scales. First, and most important, are the extremely rapid major warmings at ~13,000 and ~10,000 yr BP and the prolonged cooling trend from 12,500 to 10,500 yr BP. There are also a number of minor oscillations such as the brief periods of warming around 11,300 yr BP and between 10,000 and 8,000 yr BP. Some of these may be significant but it is likely that most are the products of the arbitrary age distribution in the sites sampled.

During the Pleniglacial (22,000-18,000 yr BP, see Fig. 2*b* for definition of chronostratigraphic terms) summer temperatures were below 10 °C and the coldest winter month around −16 °C or below. There is some suggestion on Fig. 2 that the winters were somewhat warmer around the time of maximum expansion of the British ice-sheet (20,000-18,000 yr BP) than either before or afterwards. This would be consistent with heavier snowfall.

By 14,500 yr BP the ice-sheet was in retreat or had vanished altogether in western England and Wales, and had disappeared in Scotland by ~13,000 yr BP[13]. From 14,500 until just before 13,000 yr BP the British climate was as cold as during the Pleniglacial, with the coldest winter months between −20 and −25 °C. The climate was much more continental than today, with a temperature range between warmest and coldest months of ~30-35 °C compared with 14 °C at present. Such a cold and continental thermal regime is found today in north-east European Russia and north-west Siberia. Britain today owes its mild winters to its proximity to a warm ocean. Although sea surface temperatures in the Atlantic due west of Britain are known to have been ~8 °C cooler in the summers of 14,000 years ago than today[14], this cannot account for a 20 °C cooling of the British winter. The severity of the winters, and the continentality of the climate, could be explained by extensive and continuous sea ice on the Atlantic in winter. This suggestion has already been made by Ruddiman and McIntyre[1] on the grounds of reduced ocean productivity occurring at the same time as the maximum rate of volumetric melting of glacier ice, as indicated by the isotopic composition of planktonic foraminifera. Thus, Fig. 2 gives support to Ruddiman and McIntyre's interpretation.

The period of cold climate was followed by a rapid warming of 7-8 °C in summer and ~25 °C in winter. The time-averaged data of Fig. 2*c* suggest that this took place over 800 years from ~13,300 to 12,500 yr BP, although the raw data of Fig. 2*b* suggest that the warming may have commenced as late as 13,000 and been complete by 12,700 yr BP. The timing of this event agrees very well with the sudden change from a polar to a sub-polar/transitional foraminiferal assemblage which occurred at 13,300±700 BP in core CH 73139C, 400 km west of Ireland[14]. This faunal change marks the passage of the polar front in surface waters as it migrated from its glacial position off the coast of Portugal to a position between Iceland and Greenland[1]. Detailed examination of the data used in Fig. 2 shows no evidence that the warming was any later in Scotland than in England or Wales. This suggests that the polar front migrated quickly, at least through the latitudes covered by our data.

By 12,500 yr BP the climate of England and Wales was as warm as at the present day, although still slightly more continental, with the warmest summer month reaching 17 °C, and the coldest winter month reaching 0-1 °C. No sooner had this peak of warmth been reached, however, than a cooling set in from 12,500 to 12,000 yr BP. This was followed by a 500 year period in which summer temperatures remained fairly constant. In contrast, the temperature of the coldest winter month decreased from ~ −5 °C at 12,000 yr BP to ~ −17 °C at 10,500 yr BP. The minor oscillations shown on Fig. 2*c* during this cooling may not be significant. Summer temperatures also cooled from ~11,400 yr BP onwards, reaching a minimum of ~10 °C at 10,500 yr BP. Finally, a second major warming commenced after 10,500 BP. By 9,800 yr BP the climate was as warm as it had been at the peak of the Windermere Interstadial 2,500 years earlier.

This climatic history correlates closely with the movements of the polar front reconstructed by Ruddiman and McIntyre[1]. In particular, the cold winters of the Younger Dryas (11,000-10,000 yr BP) suggest increased sea ice off the west coast of Britain and Ireland. Although Fig. 2*c* shows that the coldest winters of the Younger Dryas may have approached those of the period before 13,000 yr BP in severity, this is largely the effect of a single point ('A' on Fig. 2*b*) with a large MCR. Temperatures of −15 °C to −20 °C are more probable for the coldest part of the Younger Dryas. Ruddiman and McIntyre[1] consider the sea ice cover to have been less extensive in the Younger Dryas than in the earlier cold period before 13,000 yr BP and this may be reflected in the indication of a less continental Younger Dryas climate with slightly warmer winters. They also suggest that icebergs bearing volcanic ash drifted south-eastwards from west of Iceland towards the Bay of Biscay. Such a circulation in surface waters would also favour the accumulation of sea ice off the British coast.

Despite the good agreement between the British climatic record and the movements of the polar front[1], some discrepancies remain. The peak of the Windermere Interstadial was short-lived and during the cooling which followed (12,500-12,000 yr BP) the polar front lay far to the north of Britain. Thus, this cooling cannot have been a simple expression of movements of the polar front. A possible explanation lies in Jansen and Erlenkeuser's[15] study of the palaeoceanography of the Norwegian sea. They find that circulation like that of the present day, with deep water being formed in the Norwegian sea and flowing out into the Atlantic at intermediate depth across the Iceland-Faroes Ridge, did not begin until the start of the Younger Dryas. Before that time the circulation appears to have been reversed with Atlantic intermediate water flowing into the Norwegian sea along the upper continental slope. This pattern was established fairly suddenly at ~12,000 yr BP, although the time control on the cores studied by Jansen and Erlenkeuser is not good. Nevertheless, if correct, the proposed circulation between 12,000 and 11,000 yr BP would imply that cold surface waters flowed out of the Norwegian sea and could have cooled the ocean west of Britain. In this context we may also note the similarity in timing between the changes in atmospheric CO_2 recorded in the Dye 3 ice core[16] and both the major warming at 13,000 yr BP and the Younger Dryas cooling in Britain. However the ice core shows approximately constant CO_2 levels in the interval 13,000-11,000 yr BP. Since atmospheric CO_2 should be closely correlated with global mean temperature through the greenhouse effect, this again suggests that the cooling in Britain after the peak of the Windermere Interstadial had a local cause in the north-east Atlantic, not a global one.

Rates of climatic change can be estimated from the "most probable" temperature curves in Fig. 2*c*. The major warming events involved changes of mean annual temperature (estimated as the mean of TMAX and TMIN) of 1.7 °C per century at ~10,000 yr BP and 2.6 °C per century at ~13,000 yr BP. Because of the smoothing procedure used in the construction of Fig. 2*c*, the true rate of change may well have been faster. By taking the individual data points on Fig. 2*b* (and ignoring dating uncertainties) we obtain rates of change of 2.8 and 7.2 °C per century respectively. While undoubtedly rapid, these rates of change are only between 1.5 and 6 times faster than the rate of mean northern hemispheric warming in the first half of the twentieth century[17]. General circulation models of climatic response to atmospheric CO_2 changes show that regional changes in high latitudes could be around four times larger than the global mean response[18]. Thus, the rates of the warming events in Britain at ~13,000 and ~10,000 yr BP should not be viewed with too much surprise. They are not abnormal compared with those expected from the much smaller climatic changes of the present century. However, the late glacial changes were sustained over a period 10-20 times longer than the early twentieth century warming and were of course much larger overall.

The rates of cooling between around 12,500 and 10,500 yr BP appear to be much slower than the rates of warming. The whole climatic oscillation from glacial to interstadial to glacial conditions (14,000-10,000 yr BP) shows a saw-tooth shape which seems characteristic of other records of climatic change on the 1000-year timescale. The same shape is seen in mid-Weichselian excursions of the isotopic composition of Greenland ice cores[19] and in the isotopic composition of carbonates in Alpine lakes[20]. Following Broecker's suggestion[21] that large, rapid climatic changes might be due to the ocean-atmosphere system 'flipping' between two stable states, we note that the British palaeotemperature record is consistent with a rapid 'flip' from cold to warm conditions, but that the opposite transition must have been much more gradual. It is also noteworthy that, even ignoring the minor oscillations on Fig. 2*c*, the British climate was continuously changing from before 13,000 yr BP to some time after 9,800 yr BP. There is no period during this time when winter temperatures seem to have been stable for longer than a century or two. Summer temperatures may have been fairly constant for about 500 years after 12,000 yr BP but otherwise were also continuously changing.

Comparison with periglacial feature estimates

The annual march of monthly mean temperatures at the present day can be closely approximated by the sum of two sine waves, of which the more important has a 12-month period and accounts for over 95% of total variance[22]. In view of this, the mean annual temperature may be approximated by the mean of TMAX and TMIN. Figure 3 shows a smoothed record of mean annual temperature based on the data in Fig. 2*c*. These results, which are based entirely on the evidence provided by insect faunas, can be tested by comparing them with the threshold temperatures required for the formation of periglacial ice wedge casts and

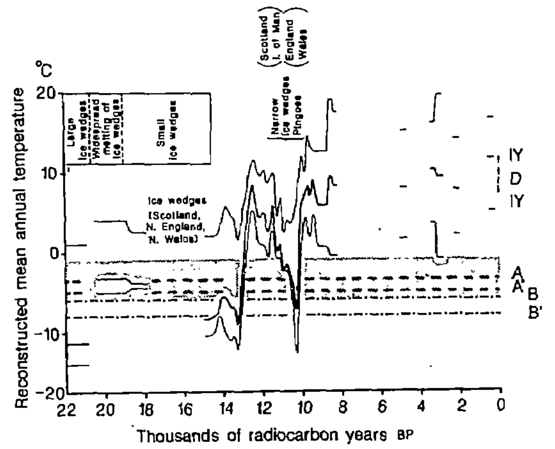

Fig. 3 Reconstructed values of mean annual temperature in England, Wales and southern Scotland, 22,000 yr BP to present, based on data in Fig. 2. The continuous fine lines show upper and lower bounds of the mutual climatic range. The bold line shows most probable value of palaeotemperature. Arrows at right show central England temperatures 1659–1980, as for Figure 2c. Shaded band shows temperature conditions within which open-system pingos can develop[25]. Broken lines, A and A', show upper temperature limit for ice wedge development according to Washburn[25] and Hamilton *et al.*[26]. Chained lines, B and B', show same limit according to Péwé[23]. Box shows occurrences of ice wedges in north-west mainland Europe[30]. Other legend shows approximate dates of periglacial features in Britain[24,27–29,31,32].

pingoes. Ice wedges have been variously described as forming where mean annual air temperatures are below −6 to −8 °C[23,24] or below −5 °C[25], or below −3.5 °C in favourable localities such as peat soils[26]. These limits are shown on Fig. 3 together with the approximate dating of ice wedge remains from Britain and the nearby parts of Europe. The coleopteran record indicates suitable conditions for ice wedges throughout the area of our data prior to 13,000 yr BP although the climate may have been only just cold enough around the time of maximum extent of the ice sheet (~20–18,000 yr BP). In fact, ice wedge casts are well known from before and after the ice sheet maximum in Britain[24,27–29]. In Europe, Kolstrup[30] records a widespread melting of large ice wedges by a date no later than 19,100 yr BP after which only smaller ice wedge casts and frost cracks occur. This is in good agreement with the warming tendency in Fig. 3 between 21,500 and 19,500 yr BP and the subsequent cooling sometime between 18,300 and 14,500 BP. Narrow ice wedge casts and cracks are also known from the Younger Dryas period (11–10,000 yr BP) in Britain, but only from Scotland[31,32] and the Isle of Man (G.R.C., unpublished data). Although Fig. 3 indicates Younger Dryas temperatures below −5 °C for 200 yr, this is largely the effect of the single value noted above ('A' on Fig. 2*b*) from St Bees Head on the mainland opposite the Isle of Man (site 8, Fig. 2*a*). Over most of the area south of Scotland, the lowest mean annual temperatures of the Younger Dryas were probably −5 °C, which is consistent with the sporadic occurrence of ice wedge casts further north.

The mean annual temperatures of −5 to −2 °C throughout most of the Younger Dryas are also consistent with the occurrence of pingo remnants in East Anglia and Wales[29], since active open system pingoes in Alaska range from −6 to −1 °C[25]. Overall, there is good agreement between the evidence of periglacial features and the mean annual temperature record based on *Coleoptera*.

Conclusion

In view of its generally good agreement with the periglacial evidence and the independent verification of the MCR method, we believe the British palaeoclimate record in Fig. 2 to be the most reliable yardstick yet produced of climatic change on the oceanic margin of north-west Europe. It is better dated than either the oceanic record or ice cores. We hope that it will provide an unambiguous point of reference in the task of unravelling the precise mechanisms by which the Earth's climate changed from a glacial to an interglacial mode, mechanisms which may well have relevance to climatic change today[21].

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Gene transfer and molecular cloning of the rat nerve growth factor receptor

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The amino-acid sequence of one form of the receptor for nerve growth factor defines a new class of growth factor receptors.

ALTHOUGH nerve growth factor (NGF) was the first growth factor to be described[1] and its physiologically relevant flow from target tissue to neuronal cell body is well characterized[2], much less is known about the transduction of signals from the NGF receptor (NGFR) or the intracellular pathways involved in mediating the functions of NGF than is the case for many mitogenic growth factors, neurotransmitters or hormones. It is known that NGF, which is required for the development of sympathetic and some sensory neurons as well as their viability in adulthood[2,3], is secreted by the target tissue near the nerve terminal, binds to a receptor and is internalized by receptor-mediated endocytosis[4,5]. The internalized NGF-containing vesicles are retrogradely transported along microtubules to the cell body[6]. Although the NGF is delivered intact and biologically active to the cell body it is quite rapidly degraded in the lysosomes[7,8]. Somewhere in this pathway the intracellular signals that are key to functions of NGF are generated.

A better understanding of what these signals are and how they are generated is likely to follow from further knowledge of the receptor or receptors for NGF. Two apparent types of NGFR exist on the NGF-responsive neurons and in PC12 cells. The major population has a lower affinity for NGF (K_d ~1 nM) and releases NGF rapidly after binding, compared to the minor population whose affinity is two orders of magnitude higher and which release NGF relatively slowly[9]. For these reasons the NGFR are identified as the fast (low-affinity) NGFR and slow (high-affinity) NGFR. Occupied slow NGFR are trypsin resistant and insoluble in Triton X-100, whereas occupied fast NGFR are trypsin labile and soluble in Triton X-100[9]. Agents which cluster the receptor, such as wheat germ agglutinin[10-12] or antibodies to NGF[12], convert the fast type to a slow type of NGFR, suggesting a relationship between the types of NGFR[13]. The slow and fast NGFR when crosslinked with ^{125}I-NGF using *N*-hydroxysuccinimidyl-4-azido-benzoate (HSAB) form complexes of relative molecular mass 158,000 and 100,000 (M_r 158K and 100K) respectively, the latter containing a single receptor peptide chain[14]. Internalization of NGF in PC12 cells uses only the slow NGFR[15,16] but both types of NGFR may be involved in this process in sympathetic neurons *in vivo*[17].

To explore the relationship between the two NGFR further and with the ultimate aim of identifying receptor-mediated intracellular signals, the complementary DNA for the fast NGFR from the rat PC12 cells has been cloned. A two-step approach was used. First, using genomic-DNA-mediated gene transfer, a set of mouse cell lines that express the rat fast NGFR were produced. A cDNA clone that coded for the rat fast NGFR was then isolated based on the difference in gene expression in the newly created NGFR$^+$ cell lines and the original mouse cell line. The 426 amino-acid sequence deduced from the cDNA sequence shows that the fast NGFR is unrelated to any other growth factor receptor. Indirect evidence suggests that the slow NGFR comprises the fast NGFR moiety together with a second subunit.

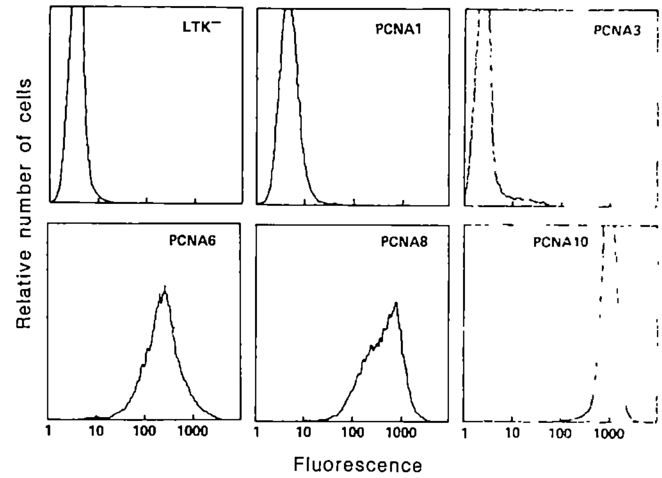


Fig. 1 Amplification of the expression of NGFR in the PCNA cell line. The first panel shows a control staining of LTK$^-$ cells. The remaining panels show the amplification of NGFR expression in the PCNA cells after repeated rounds of sorting. The number attached to the cell line name indicates the number of times the cells had been sorted.

Methods. LTK$^-$ cells (10^6) were transfected with 20 μg of PC12 genomic DNA and 1 μg of pCHTK using a modified $CaPO_4$ procedure of Wigler *et al.*[18,19]. Stable transfectants were selected by growth in hypoxanthine, aminopterin and thymidine (HAT) medium[19]. After 2-3 weeks of HAT selection the transfectants were harvested in phosphate-buffered saline (PBS) with 1 mM EDTA. Cells (10^6) were then collected by centrifugation and resuspended in 50 μl of staining medium[19] containing 1 μg of the anti-NGFR monoclonal antibody, MC192 (ref. 20). After 30 min of incubation at 4 °C the cells were washed once with 200 μl of staining medium and resuspended in 50 μl of 4 °C staining medium plus 1 μg of fluorescein-conjugated goat anti-mouse IgG and IgM (Tago, Inc.). After 20 min an equal volume of staining medium (2 ng propidium iodide ml^{-1}) was added to detect dead cells. Ten minutes later the cells were washed twice with 200 μl of staining medium. The stained cells were then resuspended in 200 μl of staining medium, passed through a 50 μm screen and sorted on a FACS. The most intensely staining cells (~1%) were collected and grown for 2-3 weeks in HAT medium at which time there were enough cells to stain and sort again.

Transfection and amplification

Mouse LTK$^-$ cells were cotransfected with high-molecular-mass rat PC12 pheochromocytoma genomic DNA (100-200 kilobases (kb) in length) and plasmid (pCHTK) containing the chicken thymidine kinase gene[18,19], and LTK$^+$ cells selected by growth in HAT medium[18]. To detect transfectants that expressed the rat fast NGFR the LTK$^+$ cells were stained with MC192, a mouse monoclonal antibody against the fast NGFR[20], and fluorescein-conjugated goat anti-mouse immunoglobulin G

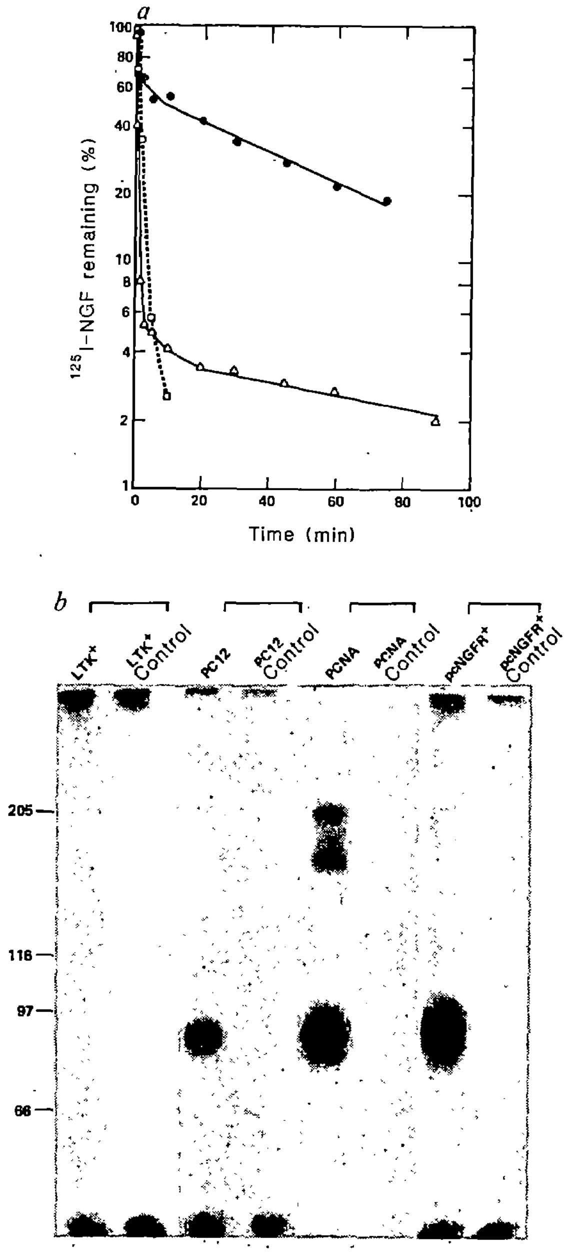


Fig. 2 Characterization of the NGFR in PC12 and transfected cell lines. *a*, Dissociation kinetics of PC12 (●), PCNA (△) and pcNGFR$^+$ (□) cells. Results are expressed as the percentage of the initial specific binding before dissociation. PCNA and pcNGFR$^+$ cells were washed once, harvested in Ca^{2+} Mg^{2+}-free PBS, 1 mM EDTA, *p*H 7.4, and washed in 10 mM HEPES/Krebs-Ringer saline, *p*H 7.4 (Krebs-Ringer), containing 1 mg ml^{-1} each of glucose and bovine serum albumin (binding buffer). PC12 cells were harvested by trituration and washed four times in binding buffer. PC12 cells (2×10^6 ml^{-1}), PCNA cells (0.5×10^6 ml^{-1}), and pcNGFR$^+$ cells (2×10^6 ml^{-1}) were incubated with 100 pM ^{125}I-NGF in 1.5 ml of binding buffer for 60 min at 22 °C, dissociation initiated by addition of 500-1000-fold unlabelled NGF at 37 °C, and residual binding assayed[9]. *b*, Determination of the size (M_r) of the NGFR. Lanes 1, 3, 5 and 7 are analyses of immunoprecipitates obtained with the MC192 antibody from LTK$^+$, PC12, PCNA, and pcNGFR$^+$ cell surfaces respectively. Control lanes are of immunoprecipitates with anti-agrin. The cells were harvested as described above, except that glucose and BSA were omitted. LTR$^+$ cells (12×10^6), PC12 cells (14×10^6), PCNA cells (13×10^6) and pcNGFR$^+$ cells (11×10^6) were surface radioiodinated[42] and solubilized in 1 ml of 0.5% Nonidet P40 Krebs-Ringer containing a mixture of 10 protease inhibitors. After one hour on ice, nuclei were removed by centrifugation at 4 °C. To one half of each supernatant 5-6 μg of anti-receptor MC192 antibody was added and the suspension was incubated at 4 °C with mixing for 12 h. The remainder (controls) received 10 μg of antibody raised against agrin (gift of Dr U. J. McMahan), After centrifugation at 10,000*g* for 10 min, 50 μl of a suspension of goat anti-mouse antibodies coupled to agarose were added to each supernatant and incubated at 4 °C with mixing for an additional 12 h. Immunoprecipitates were collected and washed four times with 0.5% NP40. After removal of excess NP40, immunoprecipitates were stored at −20 °C until analysed by SDS-polyacrylamide gel electrophoresis.[43].

(IgG). Positive cells were then isolated using a fluorescence-activated cell sorter (FACS).

In eight independent transfections, six positive cell lines were obtained. The fluorescence intensity of five of them was similar to that of PC12 cells. The sixth cell line had a heterogeneous, unstable phenotype. With repeated growth in HAT medium and sorting on the FACS the average intensity continued to increase. After 10 rounds of sorting and regrowth, a stable cell line, PCNA, was obtained (Fig. 1).

Analysis of the transfected L cell lines

The transfectants were analysed by several methods to ensure that they expressed PC12 NGFR and to determine the form of receptor. The rate of dissociation of ^{125}I-NGF from PCNA cells showed that it expressed only fast NGFR (Fig. 2*a*). Similar results were obtained with all the other transfectants. Scatchard analysis of the steady-state binding of PCNA cells revealed a single class of NGFR with a K_d (2.3 nM) identical to that of PC12 fast NGFR, and a number of NGFR (2.3×10^6 per cell) more than 10-fold greater than on PC12 cells. Analysis of the immunoprecipitates of ^{125}I-labelled PC12 and PCNA cell-surface proteins with the MC192 antibody showed the same major protein of M_r 83K (Fig. 2*b*), the expected size of the fast NGFR[14,21]. Although not apparent in this figure, the immunoprecipitate actually comprises two species with M_r s of 82K and 85K. The minor 200K species observed in these analyses is probably a disulphide-linked oligomer of the 83K species[22,23]. Finally, only the 100 K crosslinked, fast NGFR-NGF complex was observed in PCNA cells after crosslinking with HSAB. From these data we conclude that transfer of the rat fast NGFR into mouse L cells was achieved.

Cloning of the rat fast NGFR cDNA

The rat fast NGFR gene was rescued by relying on the fact that the NGFR$^+$ L-cell transfectants express fast NGFR mRNA whereas the L cells do not. In brief, a fast-NGFR-enriched probe was made by subtracting ^{32}P-labelled PCNA cDNA (made, in turn, from PCNA poly(A)$^+$ RNA) with a 20-fold excess of LTK$^+$ poly(A)$^+$ RNA[24] to remove constitutive LTK$^+$ cDNA. The remaining ^{32}P-labelled cDNA (10% of total cDNA) was used to screen 60,000 colonies of a PCNA cDNA library in pUC9[25-27]. The expression of the fast NGFR is very high in PCNA cells, so it seemed likely that the cDNA for this receptor would be one of the more abundant species detected with the subtracted

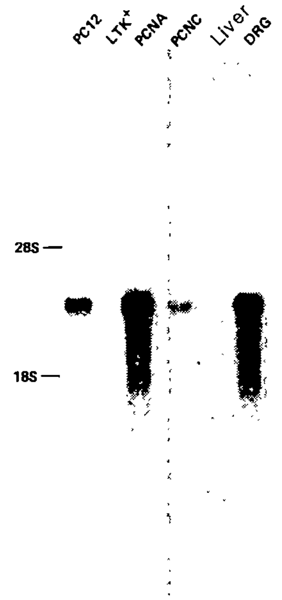


Fig. 3 Northern analysis of the NGFR cDNA clone. Total RNA[44] (10 μg) from PC12 cells, LTK$^+$ cells, PCNA cells, PCNC cells, rat liver and newborn rat dorsal root ganglia (DRG) were each separated on a 0.8% formaldehyde agarose gel[45] and blotted to a nylon membrane according to the manufacturer's instructions (Hybond, Amersham Corp.) A ^{32}P-labelled cDNA probe (~30×10^6 c.p.m.) was made from the putative NGFR cDNA clone insert (pNGFR.1), using random primers[46] and hybridized with the blot in 10 ml of 50% formamide, 5× SSPE, 5× Denhardt's, 1 mM ATP and 0.1% SDS for 3 days at 42 °C. The blot was then washed 4 times for 30 min in 0.2×SSC and 0.1% SDS at 65 °C and subjected to autoradiography[25].

robe. Based on this assumption 30 of the most intensely ybridizing colonies were picked. Rescreening reduced this umber to 19. To simplify the identification of fast NGFR clones e 19 clones were separated into groups based on cross-ybridization of their inserts. From one family of clones that eacted most strongly with the subtracted probe, a clone with n insert of 3.4 kb was selected. This insert detected a common .7-kb message in PC12, PCNA and PCNC (another transfec-nt) cells and sensory (dorsal root ganglion) neurons which xpress fast NGFR, but not in LTK$^+$ or rat liver cells which do ot (Fig. 3), suggesting that it contained a fast NGFR cDNA.

The 3.4-kb clone was inserted into the simian virus 40(SV40)-ased vector, pcDL1, in sense and antisense directions and the wo subclones (pcNGFR$^+$ and pcNGFR$^-$) independently trans-ected into LTK$^-$ cells. The FACS analysis of both LTR$^+$ cells nd the transfectant with pcNGFR$^-$ after HAT selection xhibited background levels of staining, whereas the transfec-nts with pcNGFR$^+$ stained positively. The brightest 10% of hese cells were collected, grown in HAT medium and analysed r fast NGFR in the same way as were the genomic DNA ansfectants. Scatchard analysis showed a single class of recep-rs with a K_d of 3.5 nM, similar to that of fast NGFR on PC12 nd PCNA cells, and ~17,000 receptors per cell. The rate of ^{5}I-NGF dissociation was also characteristic of the PC12 fast GFR (Fig. 2*a*) and immunoprecipitation of ^{125}-I-labelled sur-ce proteins showed the 83K species, confirming that the fast GFR in these L cells transfected with pcNGFR$^+$ is of the orrect size (Fig. 2*b*). We conclude that the 3.4-kb clone, now entified as pNGFR.1, contains the rat fast NGFR cDNA.

ucleotide sequence of fast NGFR cDNA

he nucleotide sequence of the fast NGFR cDNA, with its redicted amino-acid sequence, is shown in Fig. 5*a*. A model f the fast NGFR protein and a restriction map of the pNGFR.1 insert are given in Fig. 6. The first start codon is 114 bases from the 5′ end. The open reading frame continues for 1,275 bases which results in a precursor peptide with M_r 45,432. Preliminary amino-acid sequencing of the fast NGFR, purified from the PCNA cells, indicates that 29 N-terminal amino-acid residues are removed, leaving a lysine at the amino terminus of the mature receptor protein (T.P.M., M.J.R. & E.M.S., manuscript in preparation). The NGFR expressed by the human melanoma cell line, A875 has a nearly identical N-terminal sequence[28]. Cleavage of this signal peptide results in a mature peptide containing 396 amino-acid residues with M_r 42,478.

Analysis of the hydropathy of the predicted fast NGFR peptide[29] suggests only one plasma membrane spanning domain (residues 225-249). The amino-terminal, probably extracellular, domain of the protein, comprising 222 amino-acid residues, is very rich in cysteine residues, has 2 putative sites for *N*-linked glycosylation[30] and is very acidic. It contains four repeating elements in which the positions of the cysteine residues are highly conserved (Fig. 5*b*). The intracellular domain of 151

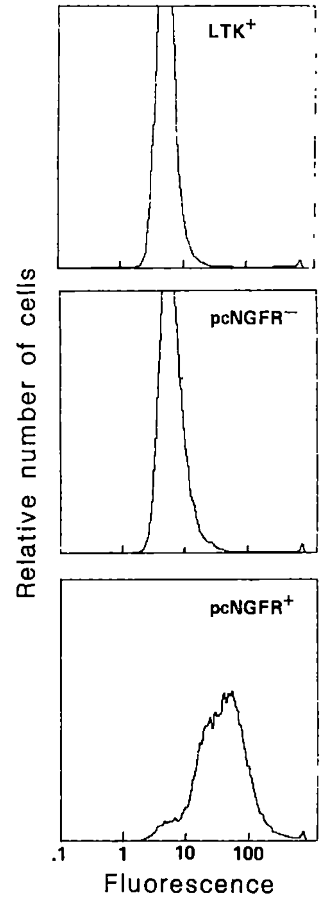
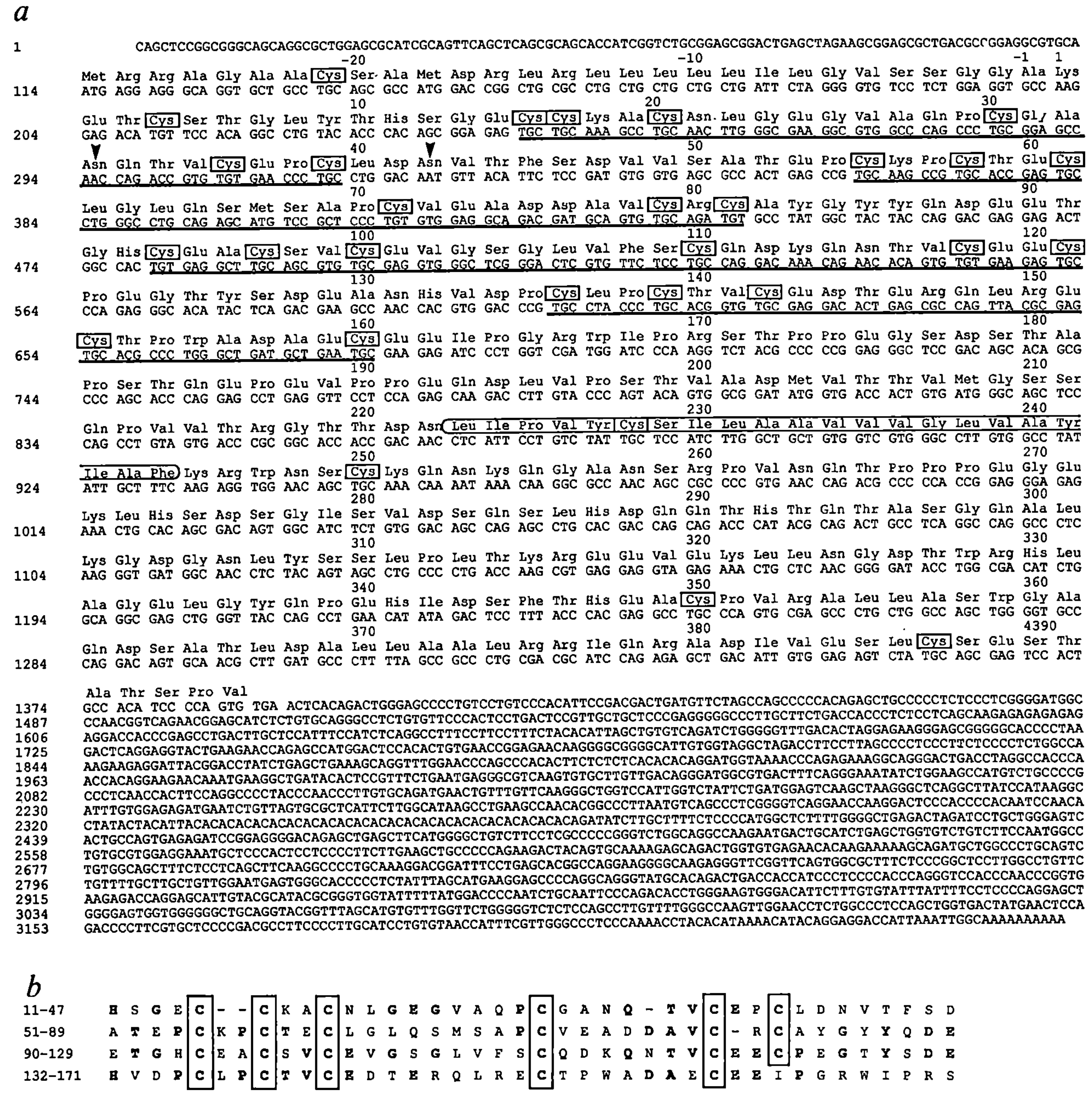


Fig. 4 Transfection of the full length NGFR cDNA in an expression vector into mouse L cells. The complete insert from the putative NGFR cDNA clone, pNGFR.1, was excised with *Eco*RI and *Hin*dIII and blunt-ended with the large fragment of *Escherichia coli* DNA Pol I[25]. The insert was then cloned in either orientation in front of the SV40 early gene promoter of pcDL1 at the *Eco*RI site which was also blunt-ended. Plasmid pcDL1, which was made from the Okayama and Berg cDNA cloning vectors pcDV1 and pcL1, was a gift of Dr Frank Lee (DNAX). An *Eco*RI site was inserted into the normal cDNA cloning site. The pcDL1-NGFR constructs (1 μg), pcNGFR$^+$ and pcNGFR$^-$, were independently transfected with 1 μg of pCHTK and 20 μg of carrier DNA (isolated from A875 cells) into LTK$^-$ cells[18,19]. LTK$^-$ cells transfected with A875 genomic DNA and pCHTK served as a control. After HAT selection the stable transfectants were stained with MC192 and FITC conjugated goat anti-mouse IgG and IgM[19]. The stained cells were then analysed using the FACS.



a

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1             CAGCTCCGGCGGGCAGCAGGCGCTGGAGCGCATCGCAGTTCAGCTCAGCGCAGCACCATCGGTCTGCGGAGCGGACTGAGCTAGAAGCGGAGCGCTGACGCCGGAGGCGTGCA
                                     -20                                     -10                                    -1   1
      Met Arg Arg Ala Gly Ala Ala Cys Ser Ala Met Asp Arg Leu Arg Leu Leu Leu Leu Leu Ile Leu Gly Val Ser Ser Gly Gly Ala Lys
114   ATG AGG AGG GCA GGT GCT GCC TGC AGC GCC ATG GAC CGG CTG CGC CTG CTG CTG CTG CTG ATT CTA GGG GTG TCC TCT GGA GGT GCC AAG
                                      10                                      20                                      30
      Glu Thr Cys Ser Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys Lys Ala Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala
204   GAG ACA TGT TCC ACA GGC CTG TAC ACC CAC AGC GGA GAG TGC TGC AAA GCC TGC AAC TTG GGC GAA GGC GTG GCC CAG CCC TGC GGA GCC
      ▼                               40      ▼                               50                                      60
      Asn Gln Thr Val Cys Glu Pro Cys Leu Asp Asn Val Thr Phe Ser Asp Val Val Ser Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys
294   AAC CAG ACC GTG TGT GAA CCC TGC CTG GAC AAT GTT ACA TTC TCC GAT GTG GTG AGC GCC ACT GAG CCG TGC AAG CCG TGC ACC GAG TGC
                                      70                                      80                                      90
      Leu Gly Leu Gln Ser Met Ser Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg Cys Ala Tyr Gly Tyr Tyr Gln Asp Glu Glu Thr
384   CTG GGC CTG CAG AGC ATG TCC GCT CCC TGT GTG GAG GCA GAC GAT GCA GTG TGC AGA TGT GCC TAT GGC TAC TAC CAG GAC GAG GAG ACT
                                      100                                     110                                     120
      Gly His Cys Glu Ala Cys Ser Val Cys Glu Val Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln Asn Thr Val Cys Glu Glu Cys
474   GGC CAC TGT GAG GCT TGC AGC GTG TGC GAG GTG GGC TCG GGA CTC GTG TTC TCC TGC CAG GAC AAA CAG AAC ACA GTG TGT GAA GAG TGC
                                      130                                     140                                     150
      Pro Glu Gly Thr Tyr Ser Asp Glu Ala Asn His Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu Arg Gln Leu Arg Glu
564   CCA GAG GGC ACA TAC TCA GAC GAA GCC AAC CAC GTG GAC CCG TGC CTA CCC TGC ACG GTG TGC GAG GAC ACT GAG CGC CAG TTA CGC GAG
                                      160                                     170                                     180
      Cys Thr Pro Trp Ala Asp Ala Glu Cys Glu Glu Ile Pro Gly Arg Trp Ile Pro Arg Ser Thr Pro Pro Glu Gly Ser Asp Ser Thr Ala
654   TGC ACG CCC TGG GCT GAT GCT GAA TGC GAA GAG ATC CCT GGT CGA TGG ATC CCA AGG TCT ACG CCC CCG GAG GGC TCC GAC AGC ACA GCG
                                      190                                     200                                     210
      Pro Ser Thr Gln Glu Pro Glu Val Pro Pro Glu Gln Asp Leu Val Pro Ser Thr Val Ala Asp Met Val Thr Thr Val Met Gly Ser Ser
744   CCC AGC ACC CAG GAG CCT GAG GTT CCT CCA GAG CAA GAC CTT GTA CCC AGT ACA GTG GCG GAT ATG GTG ACC ACT GTG ATG GGC AGC TCC
                                      220                                     230                                     240
      Gln Pro Val Val Thr Arg Gly Thr Thr Asp Asn Leu Ile Pro Val Tyr Cys Ser Ile Leu Ala Ala Val Val Val Gly Leu Val Ala Tyr
834   CAG CCT GTA GTG ACC CGC GGC ACC ACC GAC AAC CTC ATT CCT GTC TAT TGC TCC ATC TTG GCT GCT GTG GTC GTG GGC CTT GTG GCC TAT
                                      250                                     260                                     270
      Ile Ala Phe Lys Arg Trp Asn Ser Cys Lys Gln Asn Lys Gln Gly Ala Asn Ser Arg Pro Val Asn Gln Thr Pro Pro Pro Glu Gly Glu
924   ATT GCT TTC AAG AGG TGG AAC AGC TGC AAA CAA AAT AAA CAA GGC GCC AAC AGC CGC CCC GTG AAC CAG ACG CCC CCA CCG GAG GGA GAG
                                      280                                     290                                     300
      Lys Leu His Ser Asp Ser Gly Ile Ser Val Asp Ser Gln Ser Leu His Asp Gln Gln Thr His Thr Gln Thr Ala Ser Gly Gln Ala Leu
1014  AAA CTG CAC AGC GAC AGT GGC ATC TCT GTG GAC AGC CAG AGC CTG CAC GAC CAG CAG ACC CAT ACG CAG ACT GCC TCA GGC CAG GCC CTC
                                      310                                     320                                     330
      Lys Gly Asp Gly Asn Leu Tyr Ser Ser Leu Pro Leu Thr Lys Arg Glu Glu Val Glu Lys Leu Leu Asn Gly Asp Thr Trp Arg His Leu
1104  AAG GGT GAT GGC AAC CTC TAC AGT AGC CTG CCC CTG ACC AAG CGT GAG GAG GTA GAG AAA CTG CTC AAC GGG GAT ACC TGG CGA CAT CTG
                                      340                                     350                                     360
      Ala Gly Glu Leu Gly Tyr Gln Pro Glu His Ile Asp Ser Phe Thr His Glu Ala Cys Pro Val Arg Ala Leu Leu Ala Ser Trp Gly Ala
1194  GCA GGC GAG CTG GGT TAC CAG CCT GAA CAT ATA GAC TCC TTT ACC CAC GAG GCC TGC CCA GTG CGA GCC CTG CTG GCC AGC TGG GGT GCC
                                      370                                     380                                     4390
      Gln Asp Ser Ala Thr Leu Asp Ala Leu Leu Ala Ala Leu Arg Arg Ile Gln Arg Ala Asp Ile Val Glu Ser Leu Cys Ser Glu Ser Thr
1284  CAG GAC AGT GCA ACG CTT GAT GCC CTT TTA GCC GCC CTG CGA CGC ATC CAG AGA GCT GAC ATT GTG GAG AGT CTA TGC AGC GAG TCC ACT

      Ala Thr Ser Pro Val
1374  GCC ACA TCC CCA GTG TGA ACTCACAGACTGGGAGCCCCTGTCCTGTCCCACATTCCGACGACTGATGTTCTAGCCAGCCCCCACAGAGCTGCCCCCTCTCCCTCGGGGATGGC
1487  CCAACGGTCAGAACGGAGCATCTCTGTGCAGGGCCTCTGTGTTCCCACTCCTGACTCCGTTGCTGCTCCCGAGGGGGCCCTTGCTTCTGACCACCCTCTCCTCAGCAAGAGAGAGAGAG
1606  AGGACCACCCGAGCCTGACTTGCTCCATTTCCATCTCAGGCCTTTCCTTCCTTTCTACACATTAGCTGTGTCAGATCTGGGGGTTTGACACTAGGAGAAGGGAGCGGGGGCACCCCTAA
1725  GACTCAGGAGGTACTGAAGAACCAGAGCCATGGACTCCACACTGTGAACCGGAGAACAAGGGGCGGGGCATTGTGGTAGGCTAGACCTTCCTTAGCCCCTCCCTTCTCCCCTCTGGCCA
1844  AAGAAGAGGATTACGGACCTATCTGAGCTGAAAGCAGGTTTGGAACCCAGCCCACACTTCTCTCTCACACACAGGATGGTAAAACCCAGAGAAAGGCAGGGACTGACCTAGGCCACCCA
1963  ACCACAGGAAGAACAAATGAAGGCTGATACACTCCGTTTCTGAATGAGGGCGTCAAGTGTGCTTGTTGACAGGGATGGCGTGACTTTCAGGGAAATATCTGGAAGCCATGTCTGCCCCG
2082  CCCTCAACCACTTCCAGGCCCCTACCCAACCCTTGTGCAGATGAACTGTTTGTTCAAGGGCTGGTCCATTGGTCTATTCTGATGGAGTCAAGCTAAGGGCTCAGGCTTATCCATAAGGC
2230  ATTTGTGGAGAGATGAATCTGTTAGTGCGCTCATTCTTGGCATAAGCCTGAAGCCAACACGGCCCTTAATGTCAGCCCTCGGGGTCAGGAACCAAGGACTCCCACCCCACAATCCAACA
2320  CTATACTACATTACACACACACACACACACACACACACACACACACACACACACACACAGATATCTTGCTTTTCTCCCCATGGCTCTTTTGGGGCTGAGACTAGATCCTGCTGGGAGTC
2439  ACTGCCAGTGAGAGATCCGGAGGGGACAGAGCTGAGCTTCATGGGGCTGTCTTCCTCGCCCCCGGGTCTGGCAGGCCAAGAATGACTGCATCTGAGCTGGTGTCTGTCTTCCAATGGCC
2558  TGTGCGTGGAGGAAATGCTCCCACTCCTCCCCTTCTTGAAGCTGCCCCCAGAAGACTACAGTGCAAAAGAGCAGACTGGTGTGAGAACACAAGAAAAAGCAGATGCTGGCCCTGCAGTC
2677  TGTGGCAGCTTTCTCCTCAGCTTCAAGGCCCCTGCAAAGGACGGATTTCCTGAGCACGGCCAGGAAGGGGCAAGAGGGTTCGGTTCAGTGGCGCTTTCTCCCGGCTCCTTGGCCTGTTC
2796  TGTTTTGCTTGCTGTTGGAATGAGTGGGCACCCCCTCTATTTAGCATGAAGGAGCCCCAGGCAGGGTATGCACAGACTGACCACCATCCCTCCCCACCCAGGGTCCACCCAACCCGGTG
2915  AAGAGACCAGGAGCATTGTACGCATACGCGGGTGGTATTTTTATGGACCCCAATCTGCAATTCCCAGACACCTGGGAAGTGGGACATTCTTTGTGTATTTATTTTCCTCCCCAGGAGCT
3034  GGGGAGTGGTGGGGGGCTGCAGGTACGGTTTAGCATGTGTTTGGTTCTGGGGGTCTCTCCAGCCTTGTTTTGGGCCAAGTTGGAACCTCTGGCCCTCCAGCTGGTGACTATGAACTCCA
3153  GACCCCTTCGTGCTCCCCGACGCCTTCCCCTTGCATCCTGTGTAACCATTTCGTTGGGCCCTCCCAAAACCTACACATAAAACATACAGGAGGACCATTAAATTGGCAAAAAAAAAA
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b

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11-47      H S G E C - - C K A C N L G E G V A Q P C G A N Q - T V C E P C L D N V T F S D
51-89      A T E P C K P C T E C L G L Q S M S A P C V E A D D A V C - R C A Y G Y Y Q D E
90-129     E T G H C E A C S V C E V G S G L V F S C Q D K Q N T V C E E C P E G T Y S D E
132-171    H V D P C L P C T V C E D T E R Q L R E C T P W A D A E C E E I P G R W I P R S
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Fig. 5 *a*, Nucleic acid sequence of pNGFR.1 cDNA and the predicted amino-acid sequence of the rat fast NGFR. The nucleotides are numbered on the left and the amino-acid residues are numbered above the sequence. Arrows, two possible sites of asparagine-linked glycosylation; round-ended box, putative membrane-spanning domain. The cysteine residues are in rectangles and the four repreating elements are underlined. The sequence was obtained on both strands primarily using a shotgun approach[47] and dideoxy sequencing in M13mp19[48,49]. Areas of ambiguity were determined by sequencing convenient restriction fragments by the Maxam and Gilbert method[50]. The sequence of the remaining 5′ non-coding region and confirmation of the fast NGFA sequence awaits the cloning and sequencing of the genomic gene. *b*, Comparison of the sequences of the four cysteine-rich repeatng elements. The cysteines are boxed in and amino-acid residues which are conserved are in a bold type. Dashes, gaps inserted to maximize similarity.

amino acids, on the other hand, has only 3 cysteines and is essentially neutral in charge. It also lacks the consensus sequence of an ATP-binding site, a characteristic of both the tyrosine and serine or threonine kinases[31]. From its nucleotide and amino-acid sequence the fast NGFR appears to be a unique protein. Comparison with the NBR protein data bank as well as the EMBL and Genbank DNA libraries revealed no significant homologies with any other known protein in either nucleotide or amino-acid sequence. This lack of homology, especially with other growth factor receptors or oncogenes, is perhaps not surprising as NGF is a survival and differentiation factor and not a mitogen.

The difference in size between the core protein of the fast NGFR (42,478) and the mature receptor (~83 K) presumably resides in the carbohydrate moieties on one or both of the asparagine residues available for *N*-linked glycosylation and on one or more of the available *O*-linked glycosylation sites.

Comparison with other receptors

Although the fast NGFR is a unique protein it has some features in common with other receptors. Repeating elements in the cysteine-rich region in the extracellular domain are also found in other peptide-binding receptors such as the epidermal growth

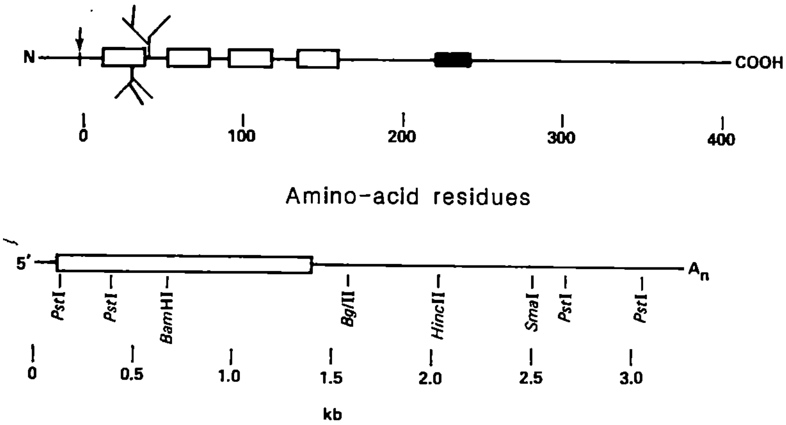

Fig. 6 Restriction map and model of the fast NGFR protein. Bottom, a representation of the transcript and its restriction map. Box, translated portion of the fast NGFR transcript; line, the untranslated portion of the cDNA. Above, a model of the fast NGFR protein. Arrow, site at which the signal peptide is cleaved; black boxes, putative membrane-spanning domain; white box, cysteine repeating elements. The two possible sites for *N*-linked glycosylation are marked by arbitrarily branched side chains.

factor (EGF)[32], insulin[33,34] and low density lipoprotein (LDL)[35] receptors. In addition, the EGF, PDGF[36] and LDL receptors, and one half of the insulin receptors, like the fast NGFR, have single membrane-spanning domains. The cytoplasmic domain is much shorter than the cytoplasmic domains of the receptors (EGF, PDGF, insulin and c-*fms*[37] receptors) with endogenous tyrosine kinase activity. It is more comparable in size to the cytoplasmic domains of the β-adrenergic (C-terminal segment)[38] and LDL receptors which also lack this activity. As in the β-adrenergic receptor the cytoplasmic domain of the fast NGFR is rich in serine and threonine residues. Some of these residues in β-adrenergic receptor are phosphorylated by a kinase only when an agonist is bound to the receptor[39]. Phosphorylation of the human fast NGFR receptor on serine and threonine residues has been reported; however the phosphorylation appears to be insensitive to NGF[21]. The fast NGFR is one of the smaller receptors to be characterized. Thus far only the interleukin-2 (IL-2) receptor, containing 251 amino-acid residues, is smaller[40,41]. This receptor is similar to the fast NGFR in that approximately half its apparent mass is carbohydrate and it displays two affinities of binding for its ligand IL-2.

The most distinctive feature of the fast NGFR compared to other growth factor receptors is the lack of an ATP binding site in the cytoplasmic domain, suggesting that the binding of NGF to the fast NGFR does not activate an endogenous kinase in the receptor. The key to the signal transduction mechanism of NGF therefore lies in the difference between the structures of the fast and the slow NGFR. Several models can be proposed to account for this difference: (1) they result from differential splicing of a common precursor mRNA; (2) they arise from separate but homologous genes; (3) they share a common NGF-binding subunit (namely the fast NGFR) or (4) they have no relation other than their ability to bind NGF. The finding that the fast NGFR cDNA hybridizes to a single mRNA species in cells (PC12 and sensory neurons) that express both types of receptor argues against the first two possibilities. It seems likely, therefore that the two receptors share the fast NGFR as a common binding subunit and that the slow NGFR contains a second subunit of $M_r \sim 60$K. The involvement of a *ras*-like gene product in the pathway of action of NGF raises the possibility that the second subunit is one of the family of G proteins.

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Note added in proof: Since submission of this article a very similar sequence has been reported for the human low-affinity NGFR (D. Johnson *et al.* *Cell* **47**, 545–554; 1986).

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Can explosions generate large-scale cosmological structure?

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The explosion model is successful in explaining certain features of galaxy formation but it is not clear *a priori* whether it can account for the large-scale distribution of galaxies. In particular, recent claims to have found 'bubbly' structure raise the question of whether explosions could have generated bubbles as large as observed. We here address this question semi-quantitatively, using analytic estimates and the results of first N-body simulations. We conclude that, in a flat Universe, the explosion model can produce a galaxy distribution on scales of a few to a few tens of megaparsecs which is not in apparent conflict with observations provided the galaxies formed at a redshift $z \approx 5$–10. If the Universe is 90% dominated by non-gaseous dark matter, the model provides a natural biased galaxy formation mechanism, as seems to be required to reconcile a flat cosmological model with observation.

The large-scale structure of the Universe is traditionally assumed to derive from primordial density fluctuations via the influence of gravity alone. However, it is also possible that the structure derives in part from non-gravitational processes, with a much weaker dependence on the initial conditions of the Universe. This is suggested, for example, by indications that galaxies did not form in the same way everywhere[1]. Such 'biasing' seems to be required if the Universe has the critical density, as suggested by the inflationary model, and it implies that some form of astrophysical feedback must have played a role in determining the large-scale structure.

A particularly interesting type of astrophysical feedback would arise if the first objects could produce explosive energy. The idea is that the shocks generated by such explosive 'seeds' (stars or clusters or stars) could sweep up background gas into dense expanding shells, which in certain circumstances would fragment into new objects. Providing radiative processes are the dominant cooling mechanism (which applies for redshifts $z < 10$), one would expect the fragments to be of galactic scale. Since the shells could have much more mass than the original seeds, this is a natural way of amplifying the scale of structure in the Universe[2-9]. If the shells eventually overlap, either before or after they have fragmented, it would also be a natural way of generating 'filaments' and 'voids'. In this paper we study this idea in some detail. First we discuss constraints on the maximum size of the individual shells and then use N-body simulations to study the effects of shell-shell interactions.

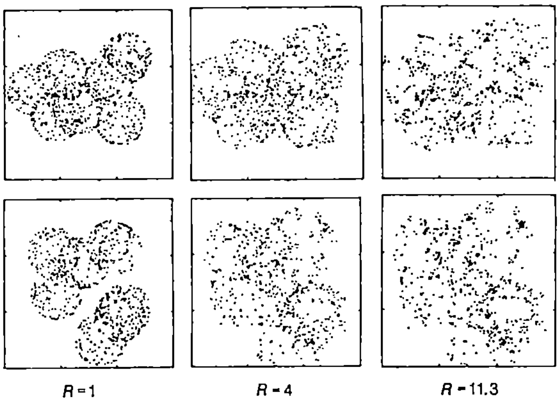

Fig. 1 Co-moving evolution (left to right) of the projected distribution of galaxies as viewed from two orthogonal directions (top and bottom) in a simulation with $\Omega_g = 0.1$.

The first constraint on the shell size derives from the requirement that the amplification factor η (the ratio of the final shell mass $M_{\rm shell}$ to the seed mass $M_{\rm seed}$) be large enough. As η decreases with increasing seed mass ($\eta \propto M_{\rm seed}^{-0.4}$), whereas $M_{\rm shell}$ increases ($M_{\rm shell} \propto M_{\rm seed}^{0.6} \propto \eta^{-1.5}$), this places an upper limit on $M_{\rm shell}$. In terms of co-moving radius normalized to the present epoch, one can show that a given value of η corresponds to a size[10]

$$R_{\rm shell} = 1.7(1+z_f)^{-0.5}\left(\frac{\varepsilon}{10^{-4}}\right)^{0.5}\left(\frac{\eta}{100}\right)^{-0.5}\left(\frac{\Omega_g}{0.1}\right)^{0.5}\Omega^{-1}\,h^{-1}\,{\rm Mpc} \tag{1}$$

where h is the Hubble constant in units of $100\,{\rm km\,s^{-1}\,Mpc^{-1}}$, z_f is the redshift at which the shell fragments, ε is the efficiency with which the seed mass is converted into explosive energy (normalized to the value associated with supernova explosions), Ω_g is the gas density in units of the critical density (normalized to the value indicated by primordial nucleosynthesis), Ω is the total density parameter (here taken to be unity), and we assume that the explosion occurs when radiative cooling dominates. If the explosive seeds are stars which also produce metals (as is most likely), η may need to exceed 100 in order to avoid generating too much background metallicity[4]. In this case, $R_{\rm shell}$ can be at most a few Mpc. Although one can weaken this constraint in various ways, even the condition $\eta > 1$ imposes a limit of a few tens of Mpc.

Another constraint derives from the requirement that the shells must be able to fragment (that is they must be able to cool). If radiative cooling dominates, one can show that this gives an upper limit on the co-moving shell radius[10] of

$$R_{\rm shell} = 3.4\,(1+z_f)^{-0.2}\left(\frac{\Delta}{4}\right)^{0.2}\left(\frac{\Omega_g}{0.1}\right)^{0.2}\Omega^{-0.6}\,h^{-0.8}\,{\rm Mpc} \tag{2}$$

where the factor Δ indicates how overdense the shell is relative to the background density. For an isolated shell, $\Delta \approx 4$ but it could be larger if shells fragment only when they collide (since this increases the shell density). Again one has a limit of about 10 Mpc.

The amplification and fragmentation constraints could be weakened if the shell fragments themselves explode. In this case, the fragments could produce a second shock which amplifies the scale of structure still further. Indeed, providing the shells do not overlap too soon, one could initiate a bootstrap process[4] (or a detonation wave[8]) in which the shell size at the present epoch could be as large as

$$R_{\rm shell} \approx 30\left(\frac{\varepsilon}{10^{-4}}\right)^{0.5} h^{-1}\,{\rm Mpc} \tag{3}$$

This is possible providing the cooling time is much shorter than the cosmological time. This condition may only be satisfied in the era when the Compton cooling of the microwave background dominates radiative cooling ($z > 10$) but, in any case, (3) gives a useful upper limit to the maximum shell size attainable in principle.

The crucial question is whether the explosion model can produce a galaxy distribution which resembles the observed one on large scales. The limited information so far available from redshift surveys indicates the presence of 'filamentary' or 'cell' structure, with thin, elongated 'superclusters' surrounding low density 'voids'[11]. The apparent scale of this structure is of the

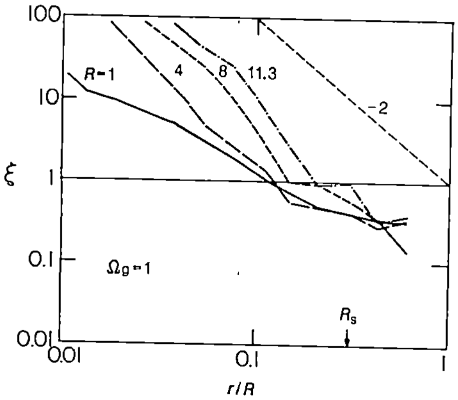

Fig. 2 Co-moving evolution of the averaged galaxy–galaxy correlation function in the scenario with $\Omega_g = 1$.

order of a few tens of megaparsecs. For example, the Local Supercluster[12] is a 'pancake' with a diameter of at least $\sim 20\ h^{-1}$ Mpc and the Perseus-Pisces Supercluster[13] is a filament with length up to $\sim 100\ h^{-1}$ Mpc. Of current interest is the distribution of galaxies in a $6° \times 120° \times 15\ h^{-1}$ Mpc slice of the Universe near Coma[14]; the observers claim to see 'bubbles' with a typical radius of $12.5\ h^{-1}$ Mpc and one especially round bubble surrounded by part of an apparently thin smooth shell of radius $25\ h^{-1}$ Mpc. A study of similar slices in the Perseus-Pisces region[15], as well as deeper surveys[16–19], also indicate the presence of filamentary structure (though not necessarily spherical 'bubbles') with a few very large voids—regions of galaxy density less than 20% of the mean—of diameter $\sim 25\ h^{-1}$ Mpc. The largest known void (in Bootes[19]) has a radius of $\sim 30\ h^{-1}$ Mpc.

If one naively interprets these voids as the interiors of the isolated shells produced in the explosion model, and the superclusters as portions of the shells themselves, then our estimates predict that the average 'void' radius would be at most $10\ h^{-1}$ Mpc and the supercluster length at most $20\ h^{-1}$ Mpc. These values are somewhat small in comparison with the observations. However, the size of the individual shells is not the whole story. Present day galaxies are certainly not distributed on the surfaces of isolated shells and this indicates that the shells, if they existed at all, must have overlapped and interacted with each other. In this case, the inter-shell interaction must have had an important effect on the characteristic scale of the final distribution. If galaxies form by spontaneous fragmentation before the shells significantly overlap, this interaction is mainly gravitational: the observed superclusters will form along the regions of overlap which may extend through several shells (see Fig. 1) and the resulting voids may be larger than one would naively expect (especially where large shells interact with smaller ones). If the shells overlap significantly while they are still gaseous, with galaxies forming only in the overlapping regions, the size of the resulting voids and superclusters is expected to be even larger. In this paper we consider only the first situation since it is easier to simulate numerically.

The simulations described below are intended to provide a preliminary study of some of the gravitational effects of the shell interactions. We focus on scales $1\text{–}20\ h^{-1}$ Mpc. At the lower end of this range, the galaxy distribution could be strongly affected by the detailed fragmentation process within the shells; we here assume a uniform random distribution along the shell surfaces. At the high end and beyond, the structure will be partially determined by the original seed distribution; this may reflect correlations due to any primordial fluctuations (for example, cold dark matter fluctuations, metric fluctuations, grain fluctuations, or fluctuations induced by cosmic strings)[21]. Here, we examined whether one can do without such primordial fluctuations, assuming that the seeds merely have a Poisson distribution initially.

Our simulations study the evolution of a gravitating system which consists of three point-like particle components, representing galaxies (gal), gas clouds (g) and dark matter clouds (DM). This is done by means of a direct N-body code which integrates the newtonian equations of motion of N particles subject to a softened gravitational potential[22]. The DM particles, which contribute Ω_{DM} to the total mass density, are distributed uniformly inside a spherical volume of radius R (set to unity at the beginning of the simulation). Prior to the explosions, the gas is also assumed to be uniformly distributed, with a density parameter $\Omega_g = 1 - \Omega_{DM}$ (the total density is taken to be critical). N_s seeds are then chosen at random positions inside the volume and a sphere of radius R_s is drawn around each of them. Each sphere is assumed to be evacuated of gas, all of this gas having fragmented at redshift z_f into galaxies which are randomly distributed on its surface. The galaxies are given an initial radial expansion speed which is 20% larger than the Hubble speed at the shell radius; this approximates the property of the cosmological similarity solution which describes the evolution of the precursor gas shells[5]. (Strictly speaking, this is an overestimate, since the similarity solution is only approached asymptotically, but the error involved is not very large.) Where the shells already happen to overlap at z_f the galaxies are placed on the plane of symmetry of each overlapping region; this simulates the result of the inelastic encounter of the two gaseous shells before z_f. In this case, we suppress the velocity component normal to the plane of intersection but retain the transverse component. The gas and the DM particles start with an unperturbed Hubble flow.

In the illustrative cases presented here, we started with 8 shells of radius $R_s = 0.3$ R, each containing 100 galaxies. The volume covered by the spheres is roughly 20% of the total volume; the precise percentage is affected by the fact that we require each sphere to be entirely contained within the radius R. The masses and initial softening radii adopted for the particles are $m = 1$, 5, 5 and $\varepsilon = 0.007\ R$, $0.035\ R$, $0.035\ R$ for the galaxies, gas and DM, respectively. The softening parameter represents the finite size of the galaxies and clouds, and is therefore fixed in the non-co-moving coordinates where R increases with time.

The first model is a purely baryonic Universe ($\Omega_g = 1$) with no DM (except perhaps for that associated with galactic haloes). The second model assumes $\Omega_g = 0.1$ and so incorporates a dominant (90%) non-baryonic DM component. We ran three realizations of each model and averaged the results. Figure 1 shows a time-sequence of the projected galaxy distribution in co-moving coordinates for the model with DM. The co-moving box size here corresponds to $2R$, that is, to 6.7 R_s. Visually, the smooth 'bubbly' structure which is apparent in the initial conditions tends to smear out with time because of gravitational clustering both within the individual shells and due to the interaction between different shells. The parts of the shells that overlap tend to become locally bound to each other: they form regions of enhanced clustering which visually resemble the observed elongated superclusters of galaxies and clusters. It seems that the superclusters mainly form in the regions of shell overlap.

These projected views are not in a form directly comparable to the 'slice' representation of de Lapparent *et al.*: the depth of each of our projections is typically $\sim 50\ h^{-1}$ Mpc (for $R_s = 10\ h^{-1}$ Mpc), while their slice corresponds to only $\sim 10\ h^{-1}$ Mpc at a distance of $100\ h^{-1}$ Mpc (where the big 'bubble' is observed). Similar slices of our simulations do show certain 'bubbly' structures but they are rather 'frothy' as the bubbles break down into smaller groups and clumps at late times. It would therefore be naive to expect round smooth bubbles to be a typical consequence of the explosion scenario. Indeed, the equivalent slices of a larger volume towards the Perseus-Pisces supercluster show a rather clumpy structure on large scales with no smooth bubbles[14].

Figure 2 shows the time evolution of the galaxy correlation function $\xi_{gal}(r)$ in the model with no DM (averaged over three

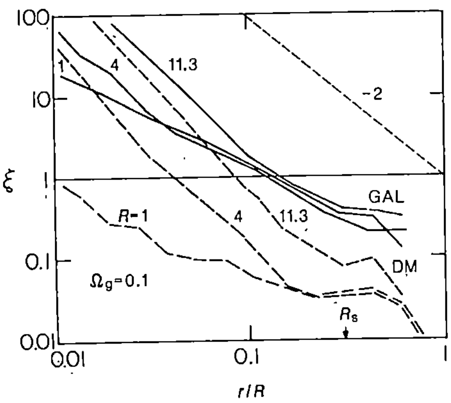

Fig. 3 Co-moving evolution of the averaged galaxy-galaxy correlation function (solid lines) against that of the dark matter (broken lines) in the scenario with $\Omega_g = 0.1$.

simulations). The quantity $1 + \xi_{gal}(r)$ is determined by counting pairs with separation in the separation interval $(r, r+\Delta r)$ and dividing this by the corresponding number of pairs in a Poisson distribution with the same number of points in the same volume (for example, ref. 23). The correlation function at the initial time looks like $\xi_{gal}(r) \propto r^{-1}$ for values of r larger than the mean galactic separation but smaller than the shell size; the correlation length (where $\xi_{gal} = 1$) is $r_c \approx 0.1\ R$. This is just what is expected[23] for a distribution of points on two-dimensional surfaces with characteristic scale and separations of a few r_c. The correlation function then steepens in time due to gravitational clustering on progressively growing comoving scales. It attains a logarithmic slope of roughly -1.8, as observed[24], at an expansion factor between $R = 4$ and $R = 8$, and it becomes even steeper afterwards. The scenario would therefore require galaxy formation at $z \approx 5$. The correlation length at this epoch, $r_c \approx 0.15\ R$, would correspond to $5h^{-1}$ Mpc, as observed[24], if $R_s = 0.3\ R$ corresponds to $10\ h^{-1}$ Mpc, so there is a rough agreement between the scale of clustering expected in the explosion model and that observed.

The model with $\Omega_g = 0.1$ is probably more realistic in view of the primordial nucleosynthesis constraints[20]. Figure 3 shows the co-moving evolution of ξ_{gal} in this case against the evolution of the DM correlation function ξ_{DM}. As before, ξ_{gal} steepens with time but it does so at a slower rate, reaching $\xi_{gal} \propto r^{-1.8}$ only at $R = 11$ (that is, galaxy formation occurs at $z = 10$)). The DM has a roughly Poisson distribution initially and so ξ_{DM} is initially very small. This introduces an initial 'bias' in the (clumped) galaxy distribution against the (smooth) DM distribution. However, the DM fluctuations grow for the following reasons: (i) the shell interiors are slowly evacuated dynamically due to the underdensity introduced by the gas within them having been swept out; (ii) the DM accretes onto the galaxies; and (iii) the initial Poissonian component of the DM fluctuations grows self-gravitationally. The white noise fluctuations introduced by the last effect resemble the cold DM fluctuations on the scales of relevance here; they become the dominant component after $R = 11$. When the galaxy and DM distributions eventually coincide, the 'bias' between them disappears. The present correlation length is again $\sim 5\ h^{-1}$ Mpc, in pleasant agreement with observations.

Apart from the slope of ξ_{gal}, an interesting constraint comes from the degree of bias, expressed for example by the ratio ξ_{gal}/ξ_{DM} at r_c; this ratio is a decreasing function of time which approaches unity at late epochs. Motivated by a desire to reconcile the elegant $\Omega = 1$ cosmological model (predicted by inflation) with the dynamics of the Local Supercluster and the cosmic virial analysis of galaxy pairs, this ratio should today be $\xi_{gal}/\xi_{DM} \approx 5$ (ref. 25 and A. Dekel, J. Einasto and M. J. Rees, in preparation). It is evident from Fig. 3 that this degree of bias indeed occurs at $R = 11$, indicating a constraint on the epoch of galaxy formation similar to that obtained on the basis of the slope of ξ_{gal}.

Finally, we make an attempt to quantify the voids, using the distribution of distances to the nearest galaxy from each point of a cubic grid[26]. We choose the grid spacing to be the mean galaxy separation, and express the distances, d, in terms of the same mean separation. Figure 4 compares the averaged histogram obtained in our simulations with the one obtained by Ryder and Turner[27] for an observed volume-limited subsample of the CfA redshift survey. The latter corresponds to the subsample limited to absolute magnitudes $M \leq -19$, which contains 489 galaxies out to a distance $r = 25\ h^{-1}$ Mpc. The histogram corresponding to the other subsample analysed by Ryder and Turner ($M \leq -21.5$, 254 galaxies, $r = 76\ h^{-1}$ Mpc), which is not shown, is in general shifted towards smaller relative separations d. The void distribution in the simulations clearly evolves in time towards larger average voids. At the epoch of galaxy formation, the theoretical voids tend to be smaller than those observed but they match each other at $R \approx 8$ in the $\Omega_g = 1$ model and at $R > 11$ in the $\Omega_g = 0.1$ model. The maximum value of d, which is an indication for the radius of the largest void, is 2.4 in the simulations; this corresponds to $13\ h^{-1}$ Mpc (for $R_s = 10\ h^{-1}$ Mpc), compared with $d = 3.4$ in the observed sample ($M \leq -19$), which corresponds to $10.4\ h^{-1}$ Mpc. (This statistic, however, should be considered with caution because it may

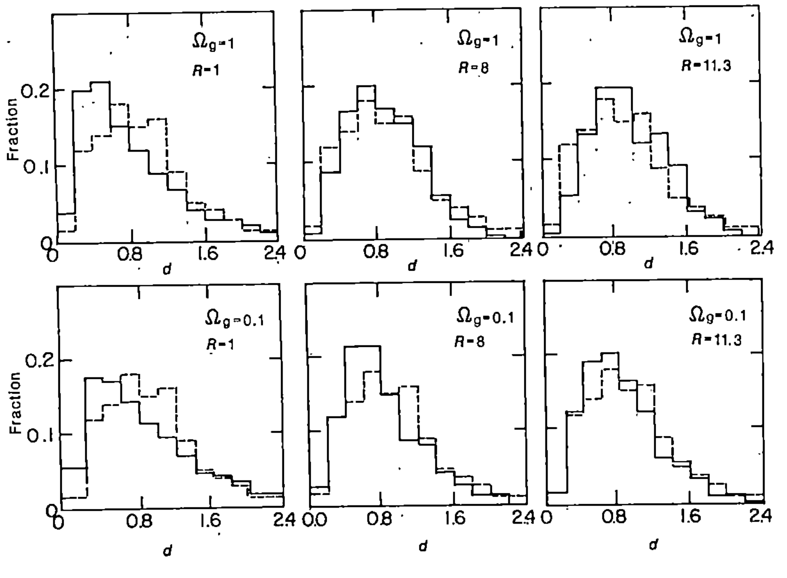

Fig. 4 Evolution (left to right) of the distribution of distances of nearest galaxies from uniform grid points in the two simulations (solid lines) compared with the results from the observed sample (broken line).

depend on the number density of galaxies in a non-trivial way, and it might be affected by edge effects.)

These results, combined with the results based on the correlation function and the visual appearance of the clustering pattern, seem to confirm the view that the large-scale distribution of galaxies in the explosion model evolves gravitationally to a distribution which roughly resembles the observed distribution at a certain time after the epoch of galaxy formation. The inferred epoch of galaxy formation is $z \approx 5$ for a baryonic Universe ($\Omega_g = 1$) and $z \approx 10$ for a Universe dominated by non-baryonic DM ($\Omega_g = 0.1$). Despite the statements sometimes made in the literature[14,28], our preliminary results indicate that it is not impossible to generate the scale of structure observed without appeal to other sources of large-scale fluctuations. Possible exceptions might be the excess clustering of rich Abell clusters[29] on scales $100\ h^{-1}$ Mpc and the existence of 'voids' with diameters exceeding $50\ h^{-1}$ Mpc. However, such features require a nontrivial explanation in all cosmogonic models currently under consideration. More extensive studies of the issues discussed here are currently in progress[10] (see D. Weinberg, A. Dekel and J. P. Ostriker, in preparation).

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Cubic ice from liquid water

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Hexagonal ice (ice Ih) is the only form of ice that is known to occur naturally on the Earth. Recently it has been suggested that small droplets of water in the upper atmosphere may often freeze first to cubic ice (ice Ic), which is metastable relative to ice Ih. The evidence is Scheiner's halo, a rare halo that occurs at ~28° from the Sun or the Moon[1–3], and the observation that dendritic snowflakes often have their c-axes at about 70° to one another[4,5]. Here we report the formation of cubic ice from liquid water at ≤200 K, using a method for vitrifying pure liquid water, namely rapid quenching of aqueous aerosol droplets ~3 μm in diameter on a cryoplate[6,7]. Cubic ice made from liquid water transforms much more slowly to the stable form than sample prepared by deposition of the vapour. Speculations about naturally occurring ice Ic (refs 1–5) are supported by our results.

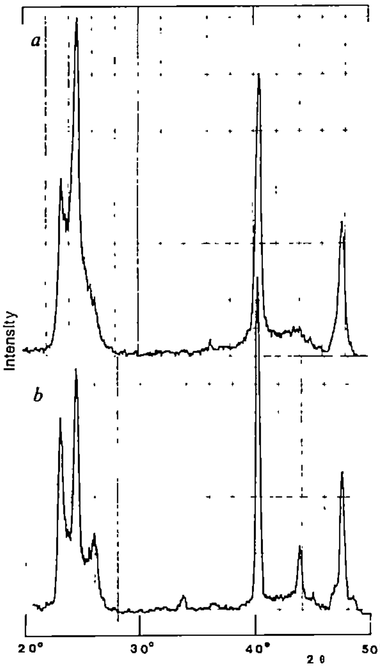

Fig. 1 X-ray diffractograms (CuKα, 100 K) of water droplets quenched (a) at 190 K and (b) at 200 K. The cubic ice samples were prepared as described in earlier reports for the vitrification of liquid water[6,7], using only higher cryoplate temperatures. Aqueous aerosol droplets, made by an ultrasonic nebulizer with ≤3 μm diameter, were transferred through a small opening, with nitrogen as carrier gas, into a high vacuum system, accelerated by supersonic flow and deposited on an X-ray sample holder acting as cryoplate. The sample holder was attached on a Displex 202 cryotip (Air Products) and the temperature regulated with the APD-E system. To obtain high cooling rate, the carrier gas has to be pumped off or condensed out. We used a second high-capacity Displex (model CS-208L) as cryopump. Deposition time was 30 min. Cryoplate tempertures were accurate at least to ±1 K. Both sample holder and deposited sample were transferred to the precooled low-temperature X-ray camera under liquid nitrogen.

This seems to be the first unambiguous report of the direct formation of cubic ice from bulk samples of pure liquid water. Earlier reports of cubic ice formation as a by-product of the vitrification of liquid water[8,9] were hampered by the fact that the low deposition temperatures (<100 K) in these experiments did not differentiate between formation from the amorphous phase and formation directly from the liquid. Likewise, the recently reported formation of cubic ice in a porous silica network[10] at 260 K is probably a result of geometric constraints of the small pores and/or of changes in the bulk properties of the liquid enclosed in the pores.

Figure 1 shows the X-ray diffractograms of water droplets quenched rapidly (a) at 190 K and (b) 200 K. The pattern in Fig. 1b indicates a mixture of ice Ic and Ih. The reflections in Fig. 1a are mainly from cubic ice. Only the 100 reflection of ice Ih (at $2\theta = 22.8°$ for CuKα) is observable as a sharp peak; the other ice Ih reflections are either very broad or absent. Similar patterns of ice Ic made from the high-pressure forms of ice have recently been analysed in terms of stacking faults[11].

The cubic ice samples were prepared using conditions where cubic ice made by deposition of water vapour is known to transform to ice Ih[12]. This demonstrates best the enhanced thermal stability of ice Ic when made from the liquid. We investigated the conversion to ice Ih in the X-ray diffractometer, using the growth of the 103 reflection (at $2\theta = 43.7°$) as an

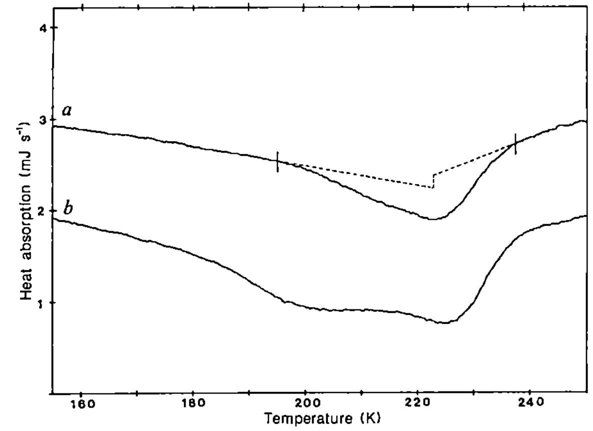

Fig. 2 Thermograms of cubic ice samples prepared by quenching of water droplets (*a*) at 190 K, and (*b*) at 170 K. The samples were investigated on a computerized Perkin-Elmer DSC-4 instrument. Heating rate was 10 K min^{-1}. Broken lines in *a* indicate the baseline chosen for the enthalpy estimate; vertical bars, the limits of integration. This type of baseline is one of the standard methods for approximating baselines with shift by extending both pre-event and post-event baselines until they connect with a line dropped vertically from the peak temperature point[15]. The mass of the water samples was determined from the heat of melting.

indicator. After 30 min at 230 K, only about 70% of ice Ic had transformed, and even at 240K similar times were necessary to obtain the final intensity of the 103 reflection.

We have in addition investigated the phase transition by differential scanning calorimetry (DSC). Figure 2*a* shows the thermogram of a cubic ice sample prepared as for Fig. 1*a*, by rapid quenching at 190 K. The broad exotherm with a peak maximum at about 223 K is due to the ice Ic→ Ih phase transition. Identical curves have been obtained from samples of vitrified liquid water prepared by quenching at 77 K. A baseline shift makes an exact evaluation of the enthalpy of transition problematic. An estimate of $-56\ \mathrm{J\,mol^{-1}}$ was obtained for a baseline shown in Fig. 2*a* with broken lines[13]. This estimate compares well with the recently determined value of $-50.5\ \mathrm{J\,mol^{-1}}$ obtained on cubic ice with small surface area[14].

In cubic ice samples prepared by quenching at 170 K, we observed an additional weak exotherm peaking between 190 and 200 K, as shown in Fig. 2*b*. It disappears after annealing at 190 K for 5 min, giving the same thermogram as in Fig. 2*a*. We ascertained by X-ray diffraction that these annealing conditions produced no hexagonal ice. However, we observed in samples quenched at 170 K that the 100 reflection of ice Ih is present only as a shoulder, and that it sharpens to a separate peak when warmed at 200 K, giving the same pattern as in Fig. 1*a*. Both effects occur in the same temperature region and might be related.

The differences in thermal stability between cubic ice made from the vapour and the liquid are probably the result of differences in surface areas. For nucleation-controlled solid-state transformations the conversion rate is strongly size-dependent[15], powders transforming more rapidly than larger crystals. It has been shown recently that ice Ic made from vapour has a large surface area[14]. For cubic ice made from water droplets, a much smaller surface area is to be expected and surface-induced nucleation should be slower. Cubic ice crystals made from water droplets in the upper atmosphere may be even more stable than our compact samples because they are isolated from one another.

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Pesticides in fog

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The discovery of the very acidic nature of fog and clouds[1,2] has created much interest in sampling, analysing, and elucidating the chemistry of fog, principally because an understanding of the chemical transformations leading to acid fog may provide important clues to the origin of acid rain. Recently, the knowledge of the chemistry of fog has expanded to include carbonyl compounds[3], volatile organic acids[4], and alkyl sulphonates[5,6]. We have discovered that a variety of pesticides and their toxic alteration products are present in fog, and that they occasionally reach high concentrations relative to reported rainwater concentrations. In our experiments, we were able to measure the air–water distribution coefficients of pesticides between the liquid fog and the interstitial gas phase. These measurements reveal that some chemicals are enriched several thousandfold in the suspended liquid fog droplets compared to equilibrium distributions expected from Henry's Law coefficients for pure aqueous solutions.

Litre-sized samples of fog liquid were collected using a high-volume, rotating stainless steel-screen collector, described elsewhere[7]. We also sampled interstitial gases and unactivated aerosol particles using a high-volume dichotomous sampler. We modified the original sampler[8] to achieve a droplet cutoff diameter of about 8 μm. Fog droplets were thus removed from the air, which was then passed first through a glass-fibre (GF) filter to trap particles, and then through a bed of porous polyurethane foam to trap vapours of pesticides and other organics[9]. The glass-fibre filter and polyurethane foam trap were extracted in a Soxhlet apparatus for 6 h using 1:1 hexane/acetone. Fog liquid was filtered through a glass-fibre filter, and filter-retained pesticides were recovered by a 12-h Soxhlet extraction with dichloromethane. The filtered fog water was extracted with dichloromethane using an established multiresidue procedure[10]. Concentrated sample extracts were split into four fractions of increasing polarity by silica gel HPLC and a hexane- to-methyl *t*-butyl ether gradient[11]. The samples were then analysed by element-selective gas chromatography, or by gas chromatography-mass spectrometry.

Fog samples were collected at the US Department of Agriculture's Beltsville Agricultural Research Center (BARC), located at Beltsville, Maryland, a suburban residential area about 15 km north-east of Washington, DC, and at various locations in the San Joaquin Valley in California. We will discuss the following four sampling events: 7 May 1984, in an area of fields and orchards on the BARC-West campus that is used for various types of experimental work; 13 January 1985 (a.m.), in the southern San Joaquin cotton-growing area near Corcoran, California; 13 January 1985 (p.m.), at the Kearney Agricultural Research Center at Parlier, California (near Fresno) in an area

Table 1 Distribution of pesticides, their alteration products, and tributylphosphate between fogwater and interstitial air

		Chemical concentration, ng l^{-1}		Distribution ratio ($\times10^6$) air–water		Enrichment factor
		fog water	Air ($\times10^3$)	measured (D)	literature (H)	EF
			-Organophosphorus insecticides-			
Diazinon	P	16,600	2.2	0.12	60	500
	C	11,800	4.2	0.36		170
	L	22,000	5.3	0.24		249
	B	140	8.6	60		1
Parathion	P	12,400	3.2	0.25	9.5	38
	C	5,800	0.88	0.15		63
	L	51,400	7.9	0.15		63
Chlorpyrifos	P	1,020	3.3	3.2	500	156
	C	320	0.6	1.9		260
	L	6,500	14.7	2.3		217
Methidathion	P	840	<0.03	<0.04	0.07	>1.8
	C	570	<0.01	<0.02		>3.5
	L	15,500	<0.6	<0.04		>1.8
Malathion	P	70	<0.03	<0.4	2.4	>6
	C	110	0.02	0.18		13
	L	350				
	B	2,740	5.2	1.9		1.3
Methyl parathion	B	1,210	7.3	6.0	4.4	0.7
			-Oxygen analogues-			
Parathion OA	P	9,000	0.21	0.023	0.25	11
	C	950	0.066	0.07		3.6
	L	184,000	0.25	0.001		250
Methidathion OA	P	120	0.03	<0.25	—	—
	L	8,200				
Chlorpyrifos OA	P	170	<0.03	<0.18	—	—
	L	800				
Diazinon OA	P	190	<0.03	<0.16	—	—
			-Cotton defoliant-			
DEF	P	250	<0.03	<0.12	320	>2,700
	C	800	0.14	0.18		1,800
			-Herbicides-			
Atrazine	P	270	<0.2	<0.7	0.2	—
	C	320	<0.16	<0.4		—
	L	700	—	—		—
	B	820	3.4	4.1		0.05
Simazine	P	390	<0.1	<0.3	0.025	—
	C	110	<0.06	<0.5		—
	L	1,200	—	—		—
	B	45	<0.04	<0.8		—
Pendimethalin	P	1,370	0.64	0.47	1,500	3,200
	C	3,620	3.6	1.0		1,500
Alachlor	B	1,450	<0.4	<0.3	1.3	>4
Metolachlor	B	1,960	<0.4	<0.2	0.37	>2
			-Flame-retardant plasticizer-			
Tributyl-phosphate	B	1,090	0.68	0.62	26	42

EF (=ratio of literature to measured distribution ratio) reflects aqueous-phase enrichment. P = Parlier, CA; C = Corcoran, CA; L = Lodi, CA.; B = Beltsville, MD.

dominated by fruit and citrus orchards; and 19 January 1985, in a dairy and grape-growing area near Lodi, California.

We found aerosol particles and non-pesticide extractable organic matter in all the fog samples. The Beltsville, Parlier, and Corcoran samples were nearly clear, but the Lodi sample was heavily loaded with particulate matter and was opaque. At most, only traces of pesticide were associated with the particles. Filtered solutions were pale yellow, with a large portion of the colour being dichloromethane-extractable organic matter. All fog liquids foamed to a certain extent, most noticeably the Lodi sample. The Beltsville sample was very acidic (pH = 2.42), the major acidic species being sulphate (SO_4^{-2}/NO_3^{-1}) ≈ 2. The California samples were much less acidic (pH range, 5.1-7.0). The inorganic chemistry of Central Valley fogs has been previously eludicated[12].

Table 1 gives the 16 pesticides and their alteration products identified in fog. As a class, the organophosphorus insecticides (diazinon, parathion, chlorpyrifos, methidathion, malathion, methyl parathion) and their oxygen analogues (oxons) were most numerous. We also found several classes of herbicides: *s*-triazines (atrazine, simazine), dinitroaniline (pendimethalin), and chloracetanilides (alachlor, metolachlor). The California fog samples yielded a greater variety and higher concentration of pesticides than Beltsville, a result attributable to the larger quantities and more diverse use of pesticides in San Joaquin Valley agriculture. Table 1 shows that the organophosphorus insecticides frequently exceeded 10 μg l^{-1} in fog, which is two to three orders of magnitude greater than commonly reported for pesticides and other neutral organics in rain from other locations[13-15]. Elevated levels of diazinon and parathion in Parlier fog may reflect local use as wintertime dormant sprays on fruit trees. This does not explain the 50 μg l^{-1} parathion found in Lodi fog, since this sample was collected in a dairy pasture area where wintertime dormant spraying is not expected.

California fog contained a number of the toxic oxygen analogues (OAs) of organophosphorus insecticides. The OA of parathion, paraoxon, was most abundant, but we also found chlorpyrifos OA, methidathion OA, and diazinon OA. Their source is uncertain. It is known that OAs form in gas-phase reactions of the parent insecticides with ozone[16,17], and also occur in high concentrations in dry surface dust[17,18], but because high oxidant levels are possible in fog, *in situ* conversion within fog droplets cannot be ruled out. OAs are potent choline esterase inhibitors and are responsible for the toxic effects of the organophosphorus insecticides[19]. This is of concern because the highest 'pesticide' concentration we measured was 184 $\mu g\, l^{-1}$ of paraoxon in Lodi fog. The laboratory-measured choline esterase inhibition of Lodi fog extracts matched that of equivalent concentrations of pure paraxon standard.

It is commonly assumed that the distribution of low solubility organics between water and air is described by Henry's Law, and that the air-to-water distribution coefficient is given by the ratio of vapour density to solubility of the pure organic chemical[13-15]. We used vapour density and solubility to calculate H as a dimensionless distribution ratio, $(ng\, l^{-1})$ vapour/$(ng\, l^{-1})$ dissolved. For those pesticides identified in fog, this calculated distribution ratio, H, ranges over five orders of magnitude: from 2.5×10^{-8} (simazine) to 1.5×10^{-3} (pendimethalin). Compounds with smaller H have a greater tendency to associate with the aqueous phase. A number of compounds with $H < 10^{-6}$ were found in fog even though their interstitial air concentrations were immeasurably low.

H was not a good quantitative estimate of the distribution of pesticides between fog droplets and air in our samples. The measured air–water distribution ratio, D, was frequently very much less than H (Table 1), especially for the California samples. $D < H$ implies an aqueous-phase enrichment: more pesticide vapour dissolves in the fog droplet than would dissolve in an ideal solution at equilibrium. The extent of this aqueous-phase enrichment is revealed by the enrichment factors ($EF = H/D$) shown in Table 1, which were large in several cases. We measured several thousandfold enrichments for DEF and pendimethalin. The reason for these high enrichments is not known, but the nature of the enrichment factors and the different conditions under which the various samples were collected rule out temperature effects and sampling artifacts.

It is remarkable how similar the EF values were for each pesticide in the three California samples, even though they were obtained under different conditions at widely separated locations. For these samples there was a relationship between EF and H: aqueous-phase enrichment increased as H increased, that is, for those pesticides which were more volatile and/or less soluble. This function, shown in Fig. 1, indicated that aqueous-phase enrichment was important in the California samples when $H > 10^{-7}$, and increased smoothly, approximately as $H^{2/3}$, over four orders of magnitude ($\log EF = 0.66\, H + 5.02$; $r^2 = 0.88$). It may be that pesticides with $H < 10^{-7}$ exhibit no aqueous-phase enrichment because of their high inherent affinity for the aqueous phase. The very high paraoxon concentration in the Lodi fog appears to be anomalous.

In addition to the data presented in Table 1, samples of eight fog events at other locations in California's Central Valley have been analysed for diazinon and parathion. For these samples, diazinon enrichment ranged from 7 to 1,100, and averaged 380; parathion enrichment ranged from 3 to 59 and averaged 20. These enrichments are consistent with those reported for Corcoran, Parlier, and Lodi in Table 1, and suggest that aqueous-phase enrichment occurs quite regularly in Central Valley fogs.

Enrichment factors for the Beltsville sample, also plotted in Fig. 1, provided an interesting contrast. Of the chemicals found in the Beltsville sample, three (alachlor, metolachlor, and tributylphosphate) were enriched in a manner similar to the California samples; three (diazinon, methyl parathion and malathion, all organophosphorus insecticides) were not enriched; and one (atrazine) had 20-fold less in the aqueous phase than expected. The grouping of classes of pesticides in this manner suggests that the phenomenon controlling enrichment results from very selective molecular or functional group interactions.

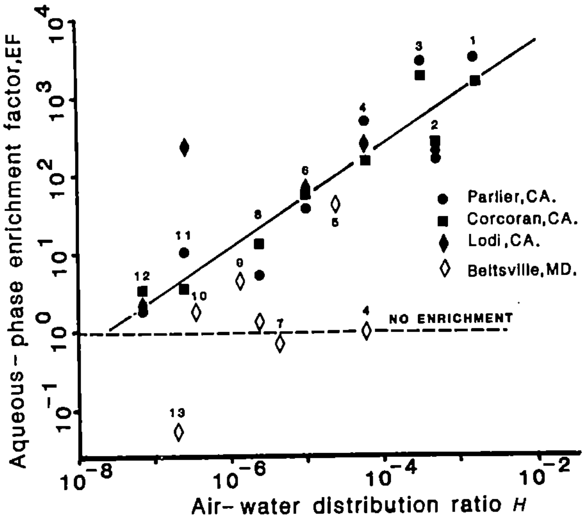

Fig. 1 Enhanced uptake of pesticides from interstitial air into fog droplets. The enrichment factor (EF, from Table 1) is the ratio of literature to measured air/water distribution ratios. 1, Pendimethalin; 2, chlorpyrifos; 3, DEF; 4, diazinon; 5, tributylphosphate; 6, parathion; 7, methyl parathion; 8, malathion; 9, alachlor; 10, metolachlor; 11, paraoxon; 12, methidathion; 13, atrazine.

Enrichment into the fog droplet was more pronounced for hydrophobic pesticides than hydrophilic ones (Fig. 1). One hypothesis to explain enrichment is that fog droplets contained solutes, such as dissolved or perhaps colloidal organic material, which increased the solubility of hydrophobic pesticides, thereby shifting the equilibrium to the solution phase. A plot similar to Fig. 1 of California-sample EF against pesticide water solubility showed that aqueous-phase enrichment generally increased with decreasing solubility, but a much poorer linear relationship resulted ($\log EF = -0.75 \log S + 2.88$; $r^2 = 0.75$). The data therefore indicate that solubility and vapour pressure are both important to the enrichment process.

Surface-active material has previously been reported in fog[20]. We know that such non-pesticidal organic matter was present in our samples, as shown by their foamy, soapy appearance. Although we have no experimental verification, it seems a reasonable conjecture that surface-active material might have been present in amounts sufficient to produce an organic film on the droplet surfaces[20]. A second hypothesis, then, is that surface-active organic matter present at the air–water interface acted to enhance the uptake of pesticides into the aqueous phase. This interpretation is consistent with the chemical-class specific enrichments found for the Beltsville fog. The very low pH of the Beltsville fog could have changed the character and interactions of fog-droplet surfactants.

Whatever the cause, it is clear there has been a shift in equilibrium, and that fog droplets are dramatically different from ideal solutions. The data show an enhanced uptake of volatile, hydrophobic organics, and suggest that non-pesticidal organics may be influencing the air–water distribution. Dissolved and colloidal organics are known to affect the solubility and air–water distribution of hydrophobic pollutants in this manner in laboratory systems[21].

Our results for the air–water distribution in fog might have implications for pesticides in other aquatic media, and particularly for other environmentally-important organics in fog. The high deposition velocity of fog droplets might represent a means of exposure for vegetation[22,23]. When fog forms, exposed plant surfaces become saturated with fog liquid for the duration of the fog event, and some organic residues may become more concentrated on the surface as moisture evaporates during fog dissipation. Also, the regional distribution of organics in fog indicated in our San Joaquin Valley samples may signal another mechanism whereby chemicals capable of entering the atmosphere may move to surfaces some distance from the target. The environmental significance of fog as a means of concentrating, moving, and depositing organic atmospheric pollutants, such as pesticides, warrants further attention.

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Upper mantle origin for Harding County well gases

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Short-lived radioisotopes such as ^{129}I and ^{244}Pu represent extremely powerful geochemical tools for tracing the early history of the Earth. Terrestrial ^{129}Xe anomalies were first found in CO_2-rich well gases from Harding County, New Mexico, and then in gases from mid-ocean-ridge basalts (MORB). Here I show that noble gases and CO_2 from Harding County are isotopically indistinguishable from such gases in fresh MORB glasses and that both probably have the same source, namely the upper mantle.

In the past 25 years, CO_2 well gases in general[1] and CO_2-rich well gases from Harding County, New Mexico, in particular, have been extensively investigated for noble gases by several authors. In 1963, Butler *et al.*[2] first found a small, but significant ^{129}Xe excess (~2%) and fissiogenic Xe excesses in CO_2-rich well gases from the Bueyeros field in Harding County (Mitchell no. 7). They interpreted such excesses to be formed by ^{129}I decay and by spontaneous fission of ^{238}U or ^{244}Pu and supposed them "to reflect the isotopic composition of deep seated terrestrial gas". Two years later, Wasserburg and Mazor[3] reported a much higher ^{129}Xe excess (8.3%) and similar fission Xe excesses in an aliquot of Mitchell no. 7. The ^{40}Ar/^{36}Ar ratios, however, range between 3,000 and 24,000 in Harding County well gases (Mitchell nos 4, 7 and 12).

Boulos and Manuel[4] in 1971 found still higher ^{129}Xe excess (9.7%) and fissiogenic Xe excesses. In 1975, Hennecke and Manuel[5] published noble-gas data on three further gas samples from Harding County, showing Xe excesses relative to air similar to those of Boulos & Manuel, ^{40}Ar/^{36}Ar ratios > 7,000, and enhanced ^{20}Ne/^{22}Ne and ^{21}Ne/^{22}Ne ratios. The most comprehensive noble-gas study on CO_2-rich well gases from the Bueyeros field, however, was carried out by Phinney *et al.*[6] in 1978. They measured the concentrations of all noble gases and the compositions of He to Xe, providing strong evidence of a primordial component in Harding County CO_2-rich well gases. As stated by Phinney *et al.*, excesses of ^{3}He and ^{129}Xe clearly show that a mantle component is present, but without a knowledge of the exact isotope signature of upper mantle noble gases, it was impossible at that time to evaluate the importance of this primitive component. In fact, CO_2-rich well gases could be a mixture of different reservoirs: atmosphere, continental crust, upper mantle.

We claim that Harding County CO_2-rich well gases represent pure noble gases derived from the upper mantle and are, except for the He component, not contaminated by gases derived from the continental crust.

In the next section, the discussion focuses on the isotope signatures of each noble gas and of carbon in CO_2.

Phinney *et al.*[6] reported a ^{4}He/^{3}He ratio of 2.28×10^5. Such a value is intermediate to that of typical MORB and atmosphere or continental crust (Table 1). Because of the low abundance of He in air, atmospheric contamination is not a likely explanation for this value, because together with atmospheric He other noble gases should be introduced into the CO_2-rich well-gas sample. A simple mass balance shows that air contamination would add more ^{36}Ar than the actual concentration in the gas sample[6]. Suppose that the He is, in fact, a mixture of MORB-type He and radiogenic He formed in the continental crust; accordingly a simple mass balance

$$(\alpha)_{CO_2} = a(\alpha)_{MORB} + (1-a)(\alpha)_{cont. crust}$$

where α is either (^{4}He/^{3}He) or (^{3}He/^{4}He), shows us that the

Table 1 Isotope noble-gas signatures

	$\frac{4}{3}$	$\frac{20}{22}$	$\frac{21}{22}$	$\frac{40}{36}$	$\frac{129}{130}$	$\frac{136}{130}$	Ref.
Continental crust	2.5×10^7	9-0.2	0.03-0.7	2,000-140,000	6.48	2.3-7.9	10, 20
CO_2-rich well gas	228,000	11.6	0.062	3,000-34,000	6.65-7.18	2.23-2.46	3-6, 12
MORB	82,000	10-13	0.03-0.07	13,000-28,000	6.6-7.5	2.25-2.55	9-11, 13, 16, 20
Atmosphere	722,500	9.8	0.029	296	6.48	2.17	21

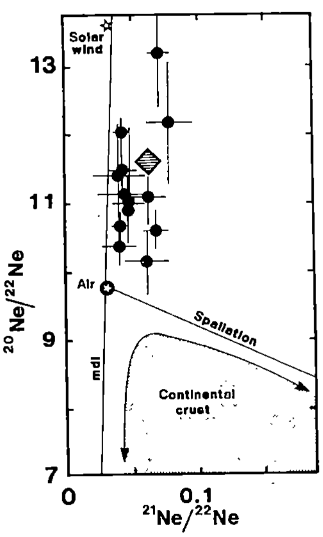

Fig. 1 Ne three-isotope plot. Included are data for MORB glasses (●)[20] and CO_2-rich well gas (◈)[6]. Also indicated are solar Ne, the mass-discrimination line for Ne (mdl), the mixing line between spallogenic Ne and atmosphere, and the field for Ne from acidic rocks[20] whose characteristics show nuclear effects of the ^{238}U-decay.

MORB-type component represents 99.4% of ^{3}He and 36% of ^{4}He, and the continental-crust component accounts for 0.6% of ^{3}He and 64% of ^{4}He.

Neon isotope systematics in CO_2-rich well gases have never been satisfactorily explained[6]. ^{20}Ne/^{22}Ne and ^{21}Ne/^{22}Ne ratios range from 9.96 (ref. 5) to 11.6 (ref. 6) and 0.0302 (ref. 5) to 0.062 (ref. 6), respectively, and are both enhanced relative to air.

Although enhanced ^{21}Ne/^{22}Ne ratios are well-known results of the nuclear reactions, for example, ^{18}O (α, n) ^{21}Ne and ^{24}Mg (n, α) ^{21}Ne, caused by U-decay[7], the same phenomenon causes low ^{20}Ne/^{22}Ne ratios (Table 1), thus defining the 'continental-crust' field in an Ne three-isotope plot (Fig. 1).

In MORB glasses, different systematics are observed. Figure 1 shows that all ^{20}Ne/^{22}Ne ratios for typical MORB glasses are above the atmospheric point. The same phenomenon has been observed for neon in diamonds[8]. The cause of this characteristic is not yet well known. Possibly, either, (1) neon from the lower mantle—with a different isotopic composition, close to solar Ne—is admixed to the MORB reservoir under the ridges, thus creating a mixing line comparable to noble gas isotope correlations reported by Allègre *et al.*[9,10] or (2) the early atmosphere lost some of its neon: through mass fractionation effects, the atmospheric point shifted down along the mass discrimination line to its present value so the variation in ^{20}Ne/^{22}Ne in MORB glasses (Fig. 1) then only represents a random distribution around a 'mantle pole'.

Through geological time, nuclear reactions due to ^{238}U-decay then enriched the MORB source in ^{21}Ne, thus shifting it to the right-hand side of the mass discrimination line. Whatever the explanation is, the CO_2-rich well gas Ne (ref. 6) is plotted perfectly in the MORB area, making any neon contribution from continental crust unlikely.

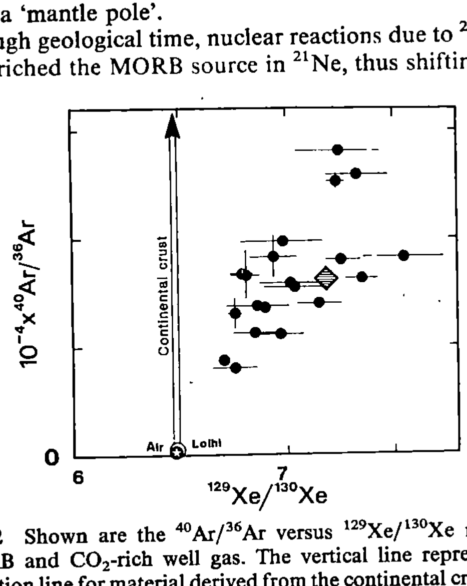

Fig. 2 Shown are the ^{40}Ar/^{36}Ar versus ^{129}Xe/^{130}Xe ratios for MORB and CO_2-rich well gas. The vertical line represents the evolution line for material derived from the continental crust. Loihi represents lower mantle material, measured by crushing under ultra-high vacuum and by stepwise heating of basalt glasses[14]. The Harding County data point is from Phinney *et al.*[6].

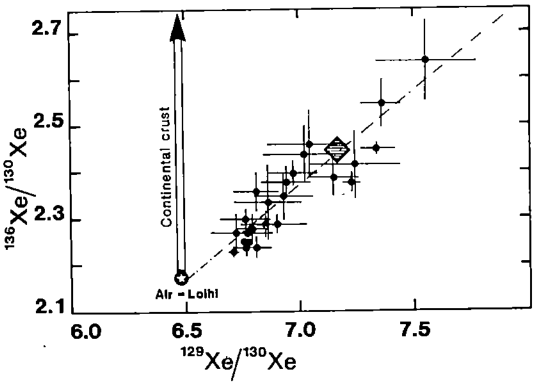

Fig. 3 ^{136}Xe/^{130}Xe versus ^{129}Xe/^{130}Xe in MORB and CO_2-rich well gas. Both the correlation between radiogenic isotopes and the excellent agreement between data for MORB and the CO_2-rich well gas are remarkable.

Zartman *et al.*[1] published ^{40}Ar/^{36}Ar ratios in CO_2-rich well gases from Harding County of 22,500 and 34,000 (Mitchell no. 4 and no. 12) whereas Phinney *et al.*[6] reported a value of 16,200. Such high values are typically observed in MORB glasses[9-11] and in felsic rocks[10]. Thus from Ar data alone, contamination by continental crust cannot be excluded, but if we combine two radiogenic isotopes, such as ^{40}Ar and ^{129}Xe (Fig. 2), then the point for the CO_2-rich well gas is within the data field of MORB samples. Any admixing of radiogenic ^{40}Ar from the continental crust should shift the point parallel to the continental crust evolution line upwards to higher ^{40}Ar/^{36}Ar ratios. This, however, is not so and Fig. 2 provides a good argument against a significant noble gas contribution from continental crust.

The best Xe data for CO_2-rich well gases are given by Hennecke and Manuel[5], Phinney *et al.*[6] and Smith and Reynolds[12]. I have chosen the data with the highest ^{129}Xe excess[6] for this comparison. Figure 3 shows ^{136}Xe/^{130}Xe versus ^{129}Xe/^{130}Xe for typical MORB glasses (refs 13, 9, 14 and Staudacher *et al.*, in preparation). Again the point for CO_2-rich well gas falls perfectly within the area defined by MORB samples. Any contribution of Xe derived from the spontaneous fission of ^{238}U from the continental crust would shift the point vertically upwards, parallel to the continental-crust evolution line. This is not so and the excellent agreement of MORB data and data for CO_2-rich well gas show that a continental-crust component in the latter is absent.

Zartman *et al.*[1] published δ^{13}C values for a large number of well gases. The CO_2-rich well gases from Harding County are quite different from most other gases, showing δ^{13}C of -3.9 and -4.1‰ (Mitchell nos 4 and 12) relative to the PDB standard. Such δ^{13}C values are typical for CO_2 within vesicles of MORB glasses[15] and confirm the similarity of the isotope signatures of CO_2-rich well gases and gases in MORB samples.

In conclusion, it can be said that CO_2-rich well gases and gases from MORB glasses show, except for He, essentially identical isotope signatures. The source region of both is probably the same. CO_2-rich well gases seemingly contain little or no gases derived from the continental crust, apart from some He. Such a He component is not surprising because of the high diffusion coefficient of He relative to other noble gases and its high concentration in continental-crustal material. For Ne, Ar, Xe and CO_2 such a continental-crustal component can be largely excluded.

Also, Phinney *et al.*[6] concluded, based on the isotopic composition of Xe from CO_2-rich well gases, that the spontaneous fission of ^{244}Pu is responsible for less than 20% of the fissiogenic

^{136}Xe excess. Because of the mantle origin of Harding County CO_2-rich well gases, this conclusion can be directly adopted for MORB noble gases.

This conclusion is important because with the published Xe data[9,10,13,16] on MORB glasses, it had not been possible to show without ambiguity if, and to what degree, the spontaneous fission of ^{244}Pu accounted for the fissiogenic Xe excesses in typical MORB glasses.

The noble-gas isotope signature for CO_2-rich well gases described above is difficult to reconcile with the conclusion by Ozima *et al.*[17] that "the source region for the ^{129}Xe excess must have been isolated for almost the whole history of the Earth from the atmosphere and/or the rest of the mantle from which the atmosphere was generated". This conclusion is based on a "xenon isotope evolution model" for the Earth as illustrated in ref. 17, Fig. 4, which uses for a model the evolution of $^{136}Xe/^{130}Xe$ and $^{129}Xe/^{130}Xe$ due to spontaneous fission of ^{244}Pu and decay of ^{129}I with the time of atmosphere outgassing (t_d) as a variable parameter. The evolution curves in their model are constrained by two points: A is the Xe isotopic composition of the present atmosphere, A_0 is the isotopic composition of the initial, non-radiogenic, non-fissiogenic atmosphere (U-Xe), estimated by Pepin and Phinney[18], which itself is largely model-dependent. In ref. 17 (Fig. 4) the data point for CO_2-rich well gas corrected for ^{136}Xe due to ^{238}U spontaneous fission falls in a forbidden area, below the dotted evolution curve ($t_d \rightarrow 0$), thus leading Ozima *et al.* to the above-mentioned conclusion.

If the source of CO_2-rich well gases or MORB was in fact isolated from the mantle from which the atmosphere was formed, then the atmosphere could not have been generated from the upper mantle, the *de facto* source of MORB. Such a hypothesis completely contradicts all known models of atmosphere formation.

If CO_2-rich well gases come from the deep mantle as Ozima *et al.* suggest, then the ^{129}Xe excesses, fission Xe excesses, high $^{40}Ar/^{36}Ar$ ratios, Ne anomalies and even radiogenic He should also come from the deep mantle. This contradicts the observations that the deep or lower mantle is less radiogenic[19,9,10,14].

A more plausible solution to this puzzle would be that the proportion of fissiogenic ^{136}Xe in the atmosphere that is due to spontaneous fission of ^{244}Pu[18] is highly overestimated and that the $^{244}Pu/^{129}I$ ratio given by Ozima *et al.* should be smaller. The U-Xe-point A_0 in ref. 17, Fig. 4 would lie higher, above $^{136}Xe/^{130}Xe = 2.1$, the evolution curves would be more flat and less curved. The '^{238}U-corrected' point for CO_2-rich well gas would agree with the 'xenon isotope evolution model', implying that the atmosphere, CO_2-rich well gases and the highly radiogenic noble gases in MORB are all generated from the same source, the upper mantle.

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Short-lived radioactive disequilibria and magma dynamics in Etna volcano

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The interest of radioactive disequilibria for the study of magmatic processes has long been recognised[1]. The use of $^{230}Th–^{238}U$ disequilibria has been growing in recent years[2–4]; but, since the pioneering work by Capaldi *et al.*[5], very few studies have been concerned with a systematic use of short-lived isotopes of the radioactive series (for example, ^{226}Ra, ^{210}Pb, ^{210}Po in the ^{238}U series, and ^{228}Ra, ^{228}Th in the ^{232}Th series). Here we present new precise results on the short-lived disequilibria in some recent lavas from Etna volcano. Our data show that a Ra enrichment took place in the Etna magma between 100 and 8,000 yr ago. Moreover, there is a clear correlation between ($^{226}Ra/^{230}Th$) activity ratios and the degree of differentiation of the magma, as indicated by its Th content. This correlation is interpreted as the result of mixing of a differentiated magma and a deep basic magma with a high ($^{226}Ra/^{230}Th$) ratio. The Ra and U enrichments relative to Th in recent volcanics could be a consequence of fluid-transfers in the mantle sources or in crustal magma reservoirs.

$^{226}Ra–^{230}Th$ and $^{228}Ra–^{232}Th$ disequilibria are of a particular interest, because both concern the same pair of chemical elements with very different half-lives for ^{226}Ra (1,600 yr) and ^{228}Ra (5.77 yr): so radioactive equilibrium will be reached at a different rate for each pair. If, for example, $(^{226}Ra/^{230}Th) = 1$ and $(^{228}Ra/^{232}Th) = 1$ in a volcanic rock (both pairs in radioactive equilibrium), this means either no fractionation at all between Ra and Th or a fractionation more than 8,000 years old. (Throughout this paper, ratios in parentheses denote activity ratios.) If $(^{226}Ra/^{230}Th) \neq 1$ and $(^{228}Ra/^{232}Th) = 1$, the Ra–Th fractionation must have occurred between 30 yr and 8,000 yr ago. If $(^{226}Ra/^{230}Th) \neq 1$ and $(^{228}Ra/^{232}Th) \neq 1$, the fractionation is less than 30 yr old. In this latter case, it is possible to date the fractionation event precisely, provided that the initial equilibrium conditions in the magma are known. The results can be discussed in a $(^{228}Ra/^{226}Ra)$ against $(^{232}Th/^{226}Ra)$ diagram, which is similar to the well-known $(^{230}Th/^{232}Th)$ against $(^{238}U/^{232}Th)$ diagram proposed by Allegre[6].

The analytical results on some recent lava flows from Mount Etna are reported in Table 1. Major element compositions of the analysed rocks are given in Table 2. All the samples are trachybasalts or basaltic hawaiites, locally known as etnaites[7]. U and Th contents have been measured by isotope dilution and mass spectrometry, $(^{230}Th/^{232}Th)$ ratios by α-spectrometry. ^{226}Ra, ^{228}Ra, ^{228}Th, ^{210}Pb have been measured by non destructive γ-spectrometry on a powdered sample of about 200 g, using a high-sensitivity (≈0.1 p.p.m. U) Ge HP(P)*γ-ray* spectrometer at the ISN (Nuclear Sciences Institut) in Grenoble[8]. More details about the method and the spectrometry system will be presented elsewhere.

The results of Table 1 show that only $(^{226}Ra/^{230}Th)$ ratios are significantly different from unity. ^{226}Ra results are in good agreement with previous data on Etna lava flows[5,9,10]. ^{228}Ra and ^{228}Th are in equilibrium with ^{232}Th in our samples, in contrast with the excesses reported by Capaldi *et al.*[5].

Table 1 U and Th contents and activity ratios of the various nuclides of the ^{238}U and ^{232}Th series

Samples	U p.p.m.	^{238}U d.p.m./g	Th p.p.m.	^{232}Th d.p.m./g	$\left(\frac{^{238}U}{^{232}Th}\right)$	$\left(\frac{^{230}Th}{^{232}Th}\right)$	$\left(\frac{^{230}Th}{^{238}U}\right)$	$\left(\frac{^{226}Ra}{^{230}Th}\right)$	$\left(\frac{^{210}Pb}{^{226}Ra}\right)$	$\left(\frac{^{228}Ra}{^{232}Th}\right)$	$\left(\frac{^{228}Th}{^{228}Ra}\right)$
1950	2.79	2.07	9.97	2.43	0.849	0.934	1.10	1.73	1.18	0.99	1.01
1964	2.50	1.85	8.96	2.19	0.847	0.943	1.12	1.88	1.00	1.02	0.99
1974	2.11	1.56	7.11	1.73	0.900	0.969	1.08	2.56	1.00	1.02	1.01
1978	2.30	1.70	7.75	1.89	0.900	0.96*	1.06	2.35	1.22	nd	nd
1979	2.52	1.87	8.81	2.15	0.868	0.947*	1.09	2.02	1.11	0.99	1.01
1983	2.41	1.79	8.29	2.02	0.882	0.935	1.06	2.23	1.20	0.98	1.04

The relative errors are estimated at 0.5% for U and Th contents (mass spectrometry), 1% for (^{238}U/^{232}Th) ratios, 1% or less for (^{230}Th/^{232}Th) ratios (1σ α counting statistics), 2% for (^{230}Th/^{238}U), 4–5% for (^{226}Ra/^{230}Th), (^{228}Ra/^{232}Th) and (^{228}Th/^{228}Ra) ratios, 10–20% for (^{210}Pb/^{226}Ra) ratios. *, Results from ref. 4. See Table 2 for a brief description of the samples.

The existence of a ^{226}Ra enrichment in volcanic rocks from Etna was first reported by Cheminée[11] in 1973. The lack of any disequilibrium between ^{228}Ra and ^{232}Th shows that the Ra–Th fractionation took place between about 30 yr and 8,000 yr ago. Moreover, as a Pb–Ra fractionation probably occurred at the time of Ra–Th fractionation, the ^{210}Pb–^{226}Ra equilibrium indicates an event older than 100 years. In other words the time of transfer of the magma between its Ra enrichment and eruption is in the range 100–8,000 years. If the Ra enrichment was a continuous process at a rate slow enough so as not to affect the ^{228}Ra–^{232}Th and ^{210}Pb–^{226}Ra equilibria, then it should have begun much more than 100 years ago.

The large Ra–Th fractionation ((^{226}Ra/^{230}Th) ≈ 2) is unlikely to be due to solid-liquid partition processes during partial melting or fractional crystallization. Such a Ra enrichment in the magma would only be possible if Ra was much more incompatible than Th ($D_{RA} \ll D_{Th}$). In solid-liquid partition processes, the behaviour of Ra is likely to be similar to that of Ba, which does not seem more incompatible than Th in Etna magmas[4]. But Ra could be a relatively mobile element, especially in the presence of fluids[11]. Ra is indeed strongly enriched in CO_2 rich carbonatite magmas[12].

The Ra enrichment in Etna may thus be the result of a contamination of the magma by fluid transfer. Such a mechanism was proposed to explain enrichments in K, Rb, Cs in some very recent lavas from Etna, mainly since the 1974 eruption[13]. Indeed the 1974, 1978, 1983 and, to a lesser extent, 1979 lavas are surprisingly rich in K, Rb, Cs compared to the more differentiated 1950 and 1964 lavas (ref. 13, and Table 2). ^{87}Sr/^{86}Sr ratios are also slightly different: 0.70345±2 for 1950 and 1964 lava flows: 0.70358±2 for 1978 and 1974 flows (unpublished results). These anomalies have been interpreted as the result of a selective contamination of the magma at depth by the sedimentary basement of Etna[13]. The samples of the 1974 and 1978 lava flows have also the highest (^{226}Ra/^{230}Th) ratios (2.56 and 2.35 respectively, see Table 1). However, the 1950 and 1964 samples still have a strong ^{226}Ra–^{230}Th disequilibrium, although they appear little affected by a contamination in alkaline elements. If this latter contamination and the Ra enrichment are the result of a common process ^{226}Ra appears to be the most sensitive tracer. Moreover, as already stated, this contamination should have occurred in the magma more than 100 yr ago and not during the intervals between successive eruptions.

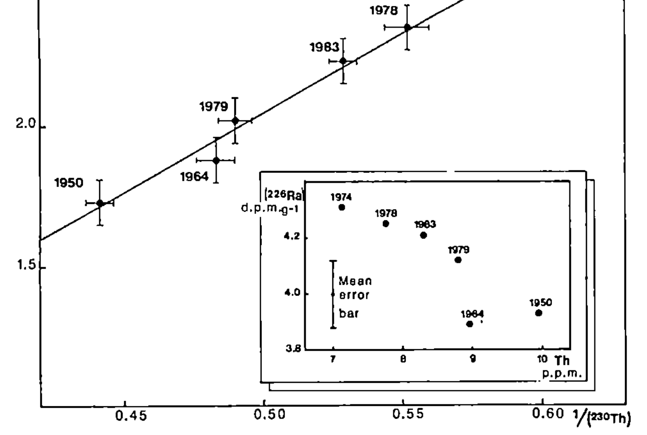

Fig. 1 Variation of (^{226}Ra/^{230}Th) ratios against 1/(^{230}Th) in recent Etna lavas. The linear correlation is interpreted by a mixing model (see text). Inset: Variation of the (^{226}Ra) activities as a function of Th content of the same samples.

It is worth noting that the variations in ^{226}Ra contents and (^{226}Ra/^{230}Th) ratios are clearly related to the degree of differentiation of the lava samples, as indicated by the good correlation between their ^{226}Ra and Th contents (Fig. 1 inset). The Ra enrichment is greater in the less differentiated lavas, as the 1974 sample, and the ^{226}Ra activities slightly decrease in the more differentiated lavas. This 1974 Ra-rich magma is likely to be close in composition to the deep Etna magma, which has already experienced some differentiation in a deep reservoir[4]. The inverse correlation between the Ra enrichment and the Th content could then result from mixing of two different magmas: a deep magma with low Th content and high (^{226}Ra/^{230}Th) ratio, and a shallow, more differentiated magma with a higher Th content and low (^{226}Ra/^{230}Th) ratio. This mixing model is strongly supported by the very good linear correlation in a (^{226}Ra/^{230}Th) against 1/(^{230}Th) diagram (Fig. 1). The basic magma and the more evolved one display also small but significant differences in their (^{230}Th/^{232}Th) and (^{238}U/^{232}Th) ratios (Table 1). The differentiated magma would have stayed in the upper part of the volcanic 'plumbing system' for a period long enough to differentiate and would have been mixed in recent years with a new basic magma rising from the deeper part of the magmatic system. This could explain the unusually intense volcanic activity of Mount Etna since 1971[7], and ^{226}Ra would be in this respect a very good tracer of basic magma reinjection. The (^{226}Ra/^{230}Th) value of a particular batch of magma erupting at the surface would depend on the relative proportions of the basic and differentiated magmas, and thus on the location and depth of its source in the volcanic 'plumbing system'. For example, the 1974 excentric eruption, which occurred at only 1,670 m above sea level, probably tapped a deep part of the volcanic feeding system and the 1974 lavas are thus the most representative of the deep magma. The subsequent eruptions tapped a 'mixed' magma from a shallower part of the volcanic system[7,14]. This explains the irregularities in the (^{226}Ra/^{230}Th) ratios variation through time.

The lower (^{226}Ra/^{230}Th) ratio of the differentiated magma can be explained in two ways: either this magma derives from a basic magma less ^{226}Ra enriched than the present one, or its (^{226}Ra/^{230}Th) ratio has decreased by radioactive decay during its stay at depth. A simple closed-system calculation, assuming

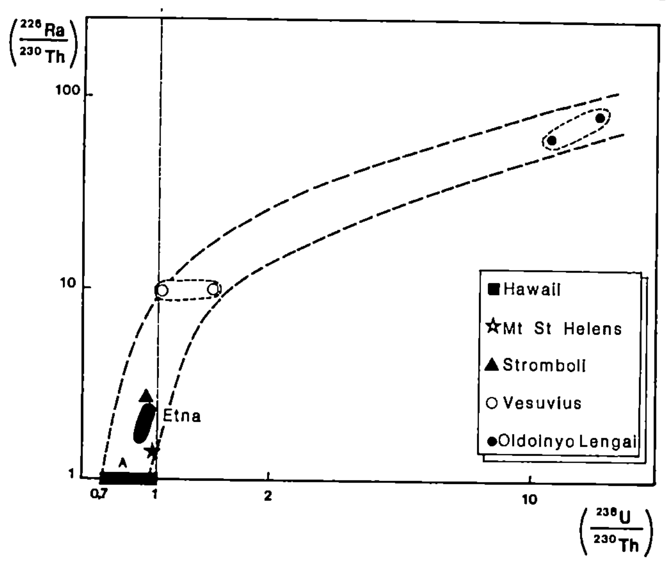

Fig. 2 Possible relationship between ($^{226}Ra/^{230}Th$) and ($^{238}U/^{230}Th$) ratios in recent lavas from different active volcanoes. High ($^{226}Ra/^{230}Th$) and ($^{238}U/^{230}Th$) ratios indicate an increasing role of fluids. The domain A shows the range in ($^{238}U/^{230}Th$) ratios of most young volcanic rocks (see text). Data from refs 12, 15, 17-19.

Table 2 Major elements analyses of the Etna samples (analyses by M. Lenoble, Laboratoire de Pétrographie, Paris 6 University)

	1950	1964	1974	1978	1979	1983
SiO_2	47.98	48.17	47.16	47.87	47.92	47.41
Al_2O_3	17.50	16.13	16.56	16.40	17.05	17.08
Fe_2O_3	5.24	3.75	4.58	4.77	3.30	3.19
FeO	5.28	7.12	6.35	6.43	7.13	7.36
MgO	4.96	5.93	5.82	5.76	5.36	5.87
CaO	10.17	10.45	11.05	10.59	10.46	10.52
Na_2O	4.31	3.94	3.56	3.66	3.97	3.81
K_2O	1.73	1.69	1.99	2.04	1.87	1.93
MnO	0.19	0.17	0.17	0.18	0.17	0.18
TiO_2	1.65	1.71	1.68	1.68	1.64	1.72
P_2O_5	0.54	0.52	0.52	0.58	0.52	0.54
H_2O^+	0.07	0.36	0.65	0.36	0.43	0.14
H_2O^-	0.13	0.03		0.03	0.10	0.06
Total	99.75	99.97	100.09	100.35	99.92	99.81

In the following description, the numbers in parentheses are those of the samples in refs 7, 14.

1950 (50-605), front of the mid-December 1950 flow, east 800 m above sea level (a.s.l.). 1964 (64-09), May 1964 south lava flow, south 3,000 m (a.s.l.). 1974 (74-1379), 25 March 1974 scoria from Mt de Fiore II, west 1,670 m (a.s.l). 1978 (78-07), April 1978 lava flow, upper part of the south-east fissure, south-east 3,000 m (a.s.l.). 1979 (79-02), front of the 6 August 1979 lava flow, east-north-east 1,780 m (a.s.l.). 1983 (83-26), 1983 lava flow, sampled on 20 June, south 2,300 m (a.s.l.).

no Ra-Th fractionation during differentiation, shows that it would take about 1,740 years to lower this ratio from 2.56 (1974) to 1.73 (1950). This is of course a maximum value since a Ra-Th fractionation produces a decrease in Ra/Th ratios.

The question remains about the origin of the Ra enrichment in the deep Etna magma. If a permanent reservoir has been present under Etna for more than 10^5 years[4], ^{226}Ra and ^{230}Th should be in equilibrium in this reservoir. Then the Ra enrichment must have occured in this reservoir, or at shallower levels, by a selective contamination induced by fluids percolating through the wall-rocks. It is also possible that a new magma was recently injected in the deep reservoir: in that case the Ra enrichment may have its source at greater depths or even in the zone of partial melting, as proposed for Vesuvius by Capaldi *et al.*[15]. If the fluids play an essential part in the Ra enrichment process, other mobile elements, such as K, Rb, Cs and, to a lesser extent, U, are likely to be enriched in the same way.

When considering the few available results on recent volcanics around the world, it is clear that the most Ra enriched products are also the most U enriched[12]. A measure of this relative enrichment compared to Th is given by the ($^{238}U/^{230}Th$) ratio. Allegre and Condomines[16] have shown that most recent volcanic rocks have ($^{238}U/^{230}Th$) ratios lower than 1 or very close to 1; there are only a few examples of volcanic rocks having ($^{238}U/^{230}Th$) ratios higher than unity: they belong either to continental high K-alkaline volcanics or to calco-alkaline volcanics; in this latter case, their U enrichment is thought to be the result of metasomatism of the mantle source through U rich fluids[16]. It is most significant that the lavas showing the largest ^{226}Ra-^{230}Th disequilibrium also have ($^{238}U/^{230}Th$) ratios higher than 1: this is the case for the carbonatites studied by Williams *et al.*[12] [($^{226}Ra/^{230}Th$) = 83, ($^{238}U/^{230}Th$) = 15] and for the Vesuvius lavas[15]. Using the available results, one may infer a rough correlation between ($^{226}Ra/^{230}Th$) and ($^{238}U/^{230}Th$) ratios (Fig. 2), which would be indicative of an increasing role of fluids towards high ($^{226}Ra/^{230}Th$) and ($^{228}U/^{230}Th$) ratios. In other words, in a 'dry' environment the magma would show ($^{238}U/^{230}Th$)<1 and ($^{226}Ra/^{230}Th$) ≈ 1, and in a 'wet' one ($^{238}U/^{230}Th$) > 1 and ($^{226}Ra/^{230}Th$) ≫ 1. A moderate role of fluids, as in Etna, would be indicated by ($^{238}U/^{230}Th$) ≤ 1 and ($^{226}Ra/^{230}Th$) > 1. Of course the composition of the fluids is likely to have an influence on the degree of Ra and U enrichment and CO_2-rich fluids, as in carbonatites, may have a predominant role.

Clearly, it is not possible at present to proceed much further in the discussion about the ultimate origin of the Ra enrichment in the Etna magma. However, we can study the kinetics of the process (continuous or episodic) by analysing old historic lava flows of well-known age. The evolution of the ($^{226}Ra/^{230}Th$)$_0$ initial ratios through time should indeed give fundamental information about magma dynamics for the past few centuries.

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Structure of the Bushveld Complex from resistivity measurements

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Knowledge of the deep structure of the arc-like bodies constituting the Bushveld Complex (South Africa) is essential not only to understand the emplacement history of this huge layered intrusion, but also for success in the exploration for the platiniferous Merensky Reef and chromitite layers occurring near its base. Despite many studies the structure of the complex has remained obscure. Here we describe new insights into the structure and additional geophysical boundary conditions, based on a geoelectrical study. This has provided a geoelectrical stratigraphy according to which a conductive layer below the granitic rocks, of both the eastern and western compartments of the Complex, is correlated with sedimentary rocks of the Transvaal Sequence over large areas, rather than with the mafic sequence as conventionally assumed. A new feature of this model is that the mafic sequence in both compartments occurs as segments of an inward-dipping sheet transgressing the layering of the sedimentary rocks into which it intruded. The geoelectrical model supports earlier gravity models in that mafic rocks are absent from the central part of the Complex, but shows that here the Bushveld granite directly overlies Archaean basement rather than Transvaal Sequence rocks as previously accepted.

The structure of the Bushveld Complex has been under scrutiny since its discovery. Cousins[1] showed that the generally accepted lopolith model[2] could not be reconciled with gravity data, and proposed an alternative model in which the Complex was thought to consist of discrete compartments. In this model the Lebowa Granite Suite (LGS)[3] in the central part of the Complex is underlain by sedimentary strata of the Transvaal Sequence and not by the heavy mafic rocks of the Rustenburg Layered Sequence (RLS)[3]. Modelling by Smit[4,5] of gravity data on the central part of the Complex follows the model proposed by Cousins[1]. Several models for the western and eastern compartments of the Complex published subsequently[6-10], based mainly on gravity data, are unanimous that the mafic RLS directly underlies the LGS, although it does not outcrop towards the centre of the ring-like structure.

Table 1 Geoelectrical stratigraphy

Geology		Resistivities in model calculations (Ω m)	Thickness in model calculations (m)
Lebowa Granite Suite (LGS)		22,000	2,250
Rustenburg Layered Suite (RLS)	upper zone	400	20,000*
	main zone	20,000	3,500
	critical zone lower zone	3,000	3,000
Transvaal Sequence	upper part of Pretoria Group	1,000-10,000	6,100
	Silverton Formation	2,000	2,000
	graphitic member of Silverton Formation	1	>1,000

* Pseudo-thickness due to anisotropic behaviour of alternating layers of conductive magnetite and resistive gabbroic rocks. The true thickness is about 2,200 m[14].

The results of ~150 deep direct current resistivity soundings on the geoelectrically ideally stratified Bushveld Complex and the older Transvaal Sequence (Fig. 1) have led to new constraints being placed on a structural model. In an otherwise electrically resistive environment, two conductive horizons—the one associated with the magnetite-bearing upper zone of the RLS and the other, a graphitic shale, situated in the Silverton Formation of the Pretoria Group, Transvaal Sequence—form ideal markers to study the structural relationships. Although both conductors have similar conductances (conductivity-thickness products), they can be distinguished by their average resistivities. Several soundings have shown that the average resistivity of the upper zone of the RLS, which contains ~24 very conductive but relatively thin magnetite layers interlayered with highly resistive gabbroic rocks, is ~400 Ω m, whereas that of the graphitic shale in the Silverton Formation is at least an order of magnitude less (Table 1, Fig. 2). Soundings on the LGS of the eastern and western compartments invariably indicate the presence of a good conductor at depth[11].

Theoretical sounding curves were calculated from average geoelectrical parameters for models, assuming the complete RLS as being either present below the LGS with decreasing dip towards the central part of the Complex (Fig. 2), or as a symmetrical intrusion with the subcrop of the RLS below the LGS (Fig. 3). These curves are displayed in Figs 2*b* and 3*b*. It is evident from these model curves that the presence of the complete RLS (Fig. 2, curve A), or even only parts of it (Fig. 3, curves B and C), below the LGS has the effect of shifting the

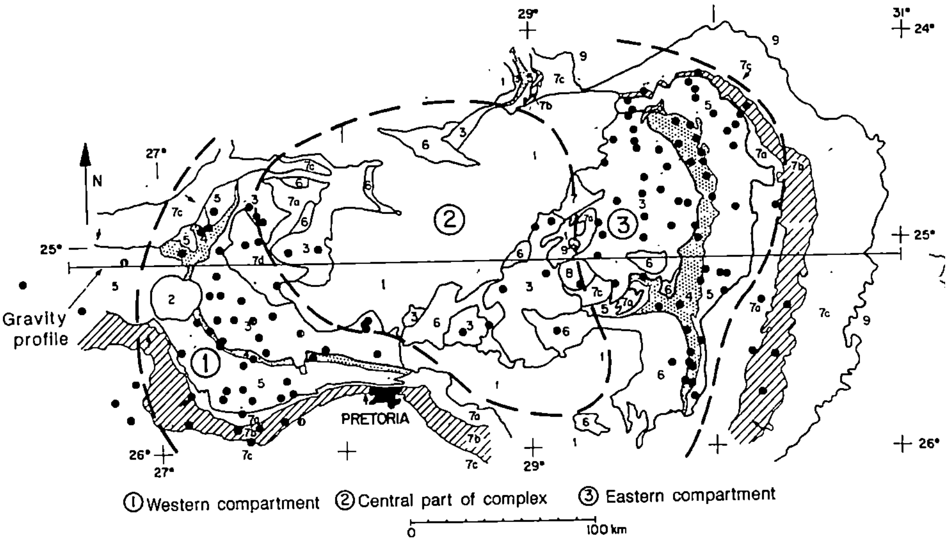


Fig. 1 Simplified geological map of the Bushveld Complex showing electrical resistivity sounding positions. Thick dashed lines indicate the boundaries of compartments and arrows indicate dip direction. The numbers represent the following geological units in order of increasing age: 1, post-Bushveld cover; 2, post-Bushveld Alkaline Complexes; 3, Lebowa Granite Suite (LGS); 4, upper zone (UZ) of Rustenburg Layered Suite (RLS); 5, main (MZ), critical (CZ) and lower zones (LZ) of RLS; 6, Rooiberg Group; 7, Transvaal—generalized (TVL); 7a, upper part of Transvaal Sequence (UTVL); 7b, Silverton Formation of Pretoria Group; 7c, lower part of Transvaal Sequence (LTVL); 7d, Crocodile River Fragment of Transvaal Sequence; 8, Elandslaagte Dome of Groblersdal Group; 9, older basement rocks.

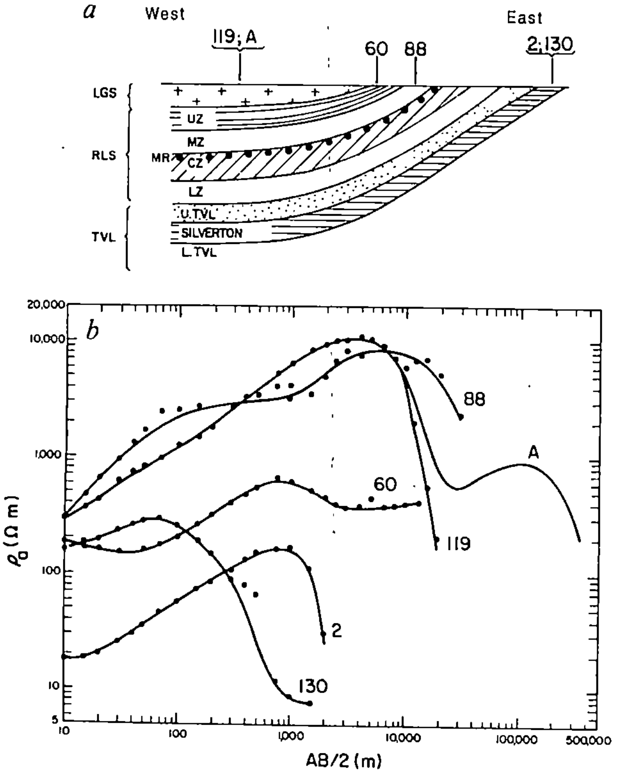


Fig. 2 A comparison of measured sounding curves (eastern compartment) with a calculated sounding curve (A) for a model in which the RLS has a gentle decreasing dip towards the centre of the Bushveld Complex. U.TVL and L.TVL refer to those formations of the Transvaal Sequence (TVL) above and below the Silverton Formation, respectively. The thickness of the LGS determined from sounding 119 was used in the calculation of curve A. *a*, Sounding positions for calculated curve (A) and field curves with assumed model; not to scale. MR, Merensky Reef (see legend to Fig. 1 for other symbols). *b*, Measured and calculated curves. Curve A was calculated according to average geoelectrical parameters established from 150 soundings.

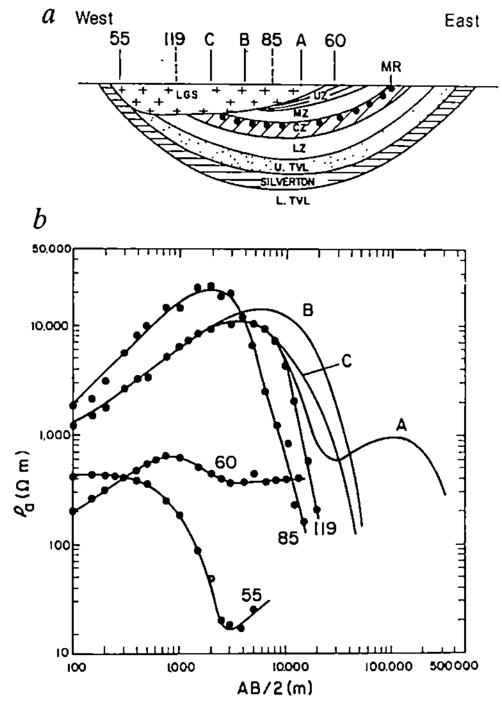


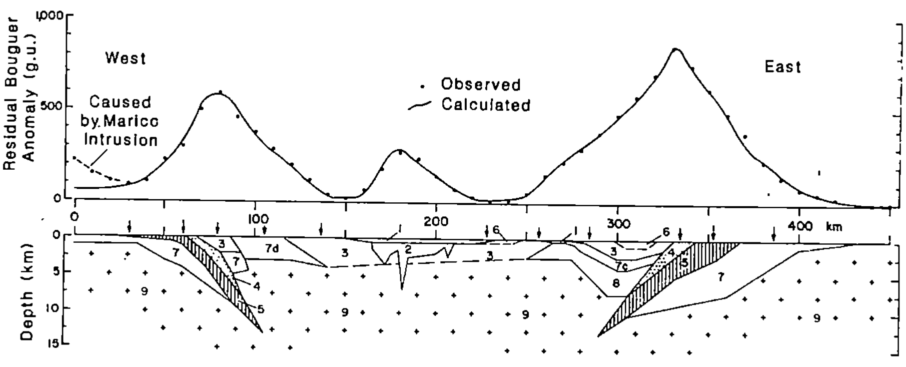
Fig. 3 A comparison of measured with calculated sounding curves for a model of the eastern compartment where the RLS is shown as a subcrop below the LGS. Feeder to the Complex not shown. *a*, Positions of measured (55, 60, 85, 119) and calculated (A, B, C) sounding curves; not to scale. Symbols as in Figs 1 and 2. *b*, Measured and calculated curves. Curves A, B and C were calculated according to average geoelectrical parameters and indicate the effect that the presence of different units of the RLS between the LGS and the Silverton Formation have on sounding curves. Sounding curves 85 and 119 are representative of the many curves measured on the LGS which all show the presence of a conductor below the resistive granite cover.

descending branch of the sounding curve farther to the right than the measured curves and causing the descent of the curve to be not as steep as those actually measured. The most pronounced difference occurs when the upper zone of the RLS is present below the LGS (curve A). Geoelectrically, the thickness of the upper zone appears to be ≥ 10 times thicker than the true thickness, owing to the anisotropic behaviour of the alternating layers of very conductive magnetite ($\rho_{\text{average}} \approx 0.07\ \Omega$ m) and resistive gabbro ($\rho_{\text{average}} \approx 10{,}000\ \Omega$ m) comprising the upper zone. None of the sounding curves so far measured on the LGS conform to any of the calculated curves for the models presented in Figs 2 and 3, and these models are therefore not representative of the structure of the Bushveld Complex.

On the other hand, the presence of a very good conductor below the LGS of the eastern and western compartments is supported by the steep descent of the final segment of all sounding curves measured on these outcrops of the LGS. This, together with the fact that the final segments of several curves have resistivities $<400\ \Omega$ m, strongly suggests that the underlying conductor reflects the presence of the Silverton Formation. At sounding 55 (Fig. 3), for example, where it is known from nearby outcrops that the LGS is underlain by and in direct contact with the Silverton Formation, the latter has a resistivity of $<15\ \Omega$ m.

From the above arguments and the similarity in geoelectrical stratigraphy for both the western and eastern compartments, we postulate that the LGS, in the central parts of these compartments, is directly underlain by rocks of the Transvaal Sequence containing the highly conductive graphitic shale member. This, however, does not exclude the possible presence in these areas of the resistive Magaliesberg Quartzite Formation between the

Fig. 4 Observed and calculated Bouguer gravity anomaly for proposed structural model across an east-west section (Fig. 1) of the Bushveld Complex. Numbers in the lower half of the figure represent the same geological units as in Fig. 1. Arrows along profile indicate the projected positions of Schlumberger soundings.



LGS and the Silverton Formation, which, in the normal stratigraphic sequence, occurs above the Silverton Formation.

This interpretation is contrary to previous gravity models[4-9] for these areas. The gravity data can, however, easily be reconciled with the geoelectrical model by treating the mafic sequence in the eastern and western compartments of the Complex as segments of an inward dipping circular sheet-like intrusion, transgressing the layering of the Transvaal Sequence at depth (Fig. 4). According to the better-constrained geoelectrical-gravity model the LGS attains a maximum thickness of 3.9 km and is directly underlain by a thick succession of Transvaal Sequence rocks in the western compartment. In the eastern compartment the LGS attains a maximum thickness of 2.6 km as determined from geoelectrical soundings[11], and is assumed to be underlain by the Transvaal Sequence resting on rocks of the older Groblersdal Group[3]. A notable disagreement between this first-order model and field geology is the reversed dips observed in the mafic rocks in a small area south-east of Groblersdal[15].

Immediately to the east of the Crocodile River Fragment (Fig. 1) in the central part of the Complex, Transvaal Sequence rocks occur below the LGS, but further to the east the resistivity results show that a resistive layer ($\rho \approx 30{,}000\ \Omega$ m), with a thickness of ~2.4 km occurs above a slightly less resistive zone ($\rho \approx 8{,}000\ \Omega$ m) overlying a less resistive basement ($\rho \approx 3{,}000\ \Omega$ m). The uppermost layer represents the LGS, whereas the second and third zones, because of their resistivity values associated with low gravity values, probably represent Archaean granitic basement. The final less resistive unit observed on the sounding curves corresponds to the medium resistivity zone present at a depth of 5-35 km below Archaean cratonic areas in southern Africa as described by Van Zijl[12]. The sharp positive Bouguer anomaly in the central part is associated with a pre-Karoo, post-Bushveld Elandskraal-type volcanic centre[13].

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Late Quaternary alluvial history of the northwestern Deccan upland region

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Continental and oceanic sediments from tropical Africa, Australia and South America have recorded analogous changes in climate and stream behaviour during the late Quaternary[1-4]. Recently, data from north-central India have provided evidence for late Quaternary changes in river regime, in response to global changes in climate in the intertropical zone[4,5]. Here we report evidence for late Quaternary river metamorphosis[6] from the northwestern Deccan upland region of India. Radiocarbon dates combined with stratigraphic data have allowed the identification of major episodes of erosion and deposition[7,8]. Supporting evidence from the Arabian sea[2,3] demonstrates that the successive phases of aggradation and incision are linked to late Quaternary climatic changes[7,8].

The landscape of the northwestern Deccan upland region is dominantly erosional and there are no known fossiliferous fluvial or lacustrine sediments of early Quaternary or Tertiary age. The Godavari, Bhima and Krishna rivers originate in the elevated high rainfall zone of the Western Ghats (700-1,400 m above sea level (ASL)) and flow through broad valleys towards the semi-arid east (Fig. 1*a*). The area is covered by Deccan traps (basalts) of Cretaceous-Eocene age. Rainfall distribution is uneven and more than two-thirds of the drainage area is in the rain shadow of the Western Ghats. The rivers are allocthonous and the peak discharge coincides with the south-west monsoon. The upland rivers are incised 5-20 m into their late Quaternary flood plains. Valley cross-sections show two alluvial fills and two river terraces[7] (Fig. 1*b*). The fluvial deposits are <5 km wide, discontinuous and generally unfossiliferous[7,8]. Stratigraphic exposures along the major rivers and borehole data have allowed us to reconstruct the late Quaternary alluvial history of the area investigated.

Two generations of fluvial deposit with quite different characteristics are apparent. The older deposits termed the 'Upper Bhima formation'[9] (UBF) are 5-35 m thick and comprise sandy-pebbly-cobbly gravel at the base, resting unconformably on basalts (Fig. 2*a*). The gravel is rich in rounded to sub-rounded clasts of basalts, quartz and chalcedony, and is cemented by calcium carbonate. The gravel is crudely bedded, clast-supported and represents near-channel deposits[7]. This litho-unit is disconformably overlain by yellow brown or reddish brown sandy silts (silt+clay = 40-60%). Lenticular patches of sands and sandy-pebbly gravel intergrade with the silts in the lower part of the unit (Fig. 2*a*). In general, the silts and loams are laminated to massive, while planar cross-stratification characterize coarser beds. Calcareous bands of groundwater origin and nodules and tubules of pedogenic origin, are common in this formation. Fissured clays varying in thickness from 0.2 to 5.0 m also occur as channel pool deposits[7] in association with the gravel and silt. The uppermost sandy-silty unit (2-10 m thick) is generally massive and contains lenses of channel sand and colluvial gravel. The younger formation (2-15 m thick), inset against the older one and designated as 'post-black soil formation'[9] (PBF), is represented by non-calcareous dark brown sandy-silts, with gravelly lenses at the base. This formation exhibits fine alternations between sands and silts and represents overbank accretion[7] (Fig. 2*b*). Texturally, the PBF is finer than the UBF. Laboratory studies of the finer sediments of both PBF and UBF show that these sediments are alkaline (*p*H 7-9) and are rich in minerals like plagioclase, augite, magnetite and ilmenite. The clay fraction is dominated by montmorillonite and minerals of the beidellite group[8] and the fine sediments of the PBF appear to be redeposited UBF sandy silts.

The UBF and the PBF, although generally lacking fossils, have yielded animal and plant fossils and abundant archaeological material[7-10]. The basal coarse member of the UBF contains Acheulian and middle Palaeolithic artefacts, wood, and animal fossils (Fig. 2*a*)[8-10]. Faunal remains of *Bos* species *Elephus* species and *Hippopotamus* species have been found in this litho-unit[8-10]. The upper fine member of this formation has yielded Upper Palaeolithic and Epi-palaeolithic artefacts and fossilized animal bones and wood[8-10]. The PBF in comparison, has yielded drift wood and bones (animal and human), potsherds and stone artefacts of Neolithic-Chalcolithic age[8-10].

Radiocarbon dates from different sites within the study area (Fig. 1*a*)[7,11,12] provide some indications of the probable age of the alluvial deposits (Table 1). Relative dates, based on fluorine-

Table 1 Carbon-14 dates for charcoal, wood, shells and bone from the upland region of northwestern Deccan (India)

	Rivers	Lab. No.	Material	^{14}C date yr BP
PBF	Ghod	TF-1330	wood	3,175 ± 105
	Godavari	PRL-77	charcoal	3,400 ± 115
	Godavari	PRL-384	charcoal	3,630 ± 100
	Krishna	TF-1213	shells	3,855 ± 710
	Sina	BS-458*	wood	4,260 ± 110
	Nandi	BS-417	shells	7,150 ± 80
UBF u.m.	Krishna	TF-1178	shells	10,035 ± 125
	Mula	TF-1111	bone	10,310 ± 155
	Ghod	BS-146	shells	11,700 ± 150
	Pravara	BS-576	shells	12,850 ± 190
	Pravara	PRL-470	shells	12,890 ± 350
	Pravara	BS-575	shells	16,420 ± 260
	Godavari	TF-891†	shells	17,075 ± 660
	Ghod	TF-1177	shells	19,290 ± 360
	Ghod	TF-1003	shells	$21{,}725^{+630}_{-585}$
UBF l.m.	Agran	BS-661	shells	21,780 ± 460
	Pravara	BS-78	shells	24,670 ± 170
	Godavari	BS-163	shells	26,635 ± 425
	Godavari	BS-662	shells	28,390 ± 980
	Mula	TF-345	wood	30,030 ± 5715
	Krishna	TF-1004	shells	$38{,}480^{+8940}_{-4125}$
	Mula	TF-217	wood	>39,000
	Ghod	BS-43	wood	39,010 ± 3215

Remaining dates after Deccan College, Pune. u.m., Upper fine member; l.m., lower coarse member.

* ref. 11; † ref. 12.

phosphate ratio (100 F/P_2O_5)[13] on over a dozen bones, support the radiocarbon dates. Much of the exposed UBF belongs to the late Pleistocene including last glacial maximum, and ranges in age from ~>30,000 to ~10,000 yr BP. The basal gravel of the UBF is beyond the range of C-14 dating. However, the occurrence of early and late Acheulian artefact assemblages in this unit suggests a late-middle Pleistocene age[7]. Fluorine-phosphate dating of the associated fossil fauna has given values ranging between 6 and 8 which are comparable with the late-middle Pleistocene bones from Narmada and other parts of the Deccan[13]. The younger formation (PBF) is of late Holocene age (~4,500 to ~3,000 yr BP). The Neolithic-Chalcolithic cultural remains and relative dates on bone further support this inferred age.

Periods of aggradation and degradation during the late Quaternary are immediately recognizable from Table 1. It appears that near-equilibrium conditions have prevailed for only limited periods of time during the last ~>30,000 yr. Major aggradation occurred between ~>30,000 and 10,000 yr BP, with deposition of fine sediments between ~17,000 and 10,000 yr BP, coevally with colluvial sediments and marginal aeolian deposition of loess in the foothills, away from the banks[14]. Incision took place between ~10,000 and 4,500 yr BP, followed by deposition of a lower magnitude, from ~4,500 to ~3,000 yr BP. Since then, incision with intermittent deposition have dominated the fluvial regime[7,14].

On the basis of Schumm's[15] classification of alluvial channels and the modern channel data from the upland region, two distinct channel-fill types can be recognised within the deposits, namely, bedload and suspended load channels. The suspended load channels, dominated by silt-clay deposits, were narrow and in most cases sinuous[7,14]. Lateral migration of these meandering channels up to 300 m during the end-Pleistocene is clearly demonstrated by the borehole data[14]. The coarse member, in contrast, reflects deposition by shallow, wide, non-meandering, seasonally high discharge bed-load streams[7,14]. The basal coarse member of the UBF is overlain by overbank deposits, indicating river transformation (metamorphosis) from bedload streams (~>30,000-17,000 yr BP) to suspended load channels during the closing phase of the Pleistocene. The textural and stratigraphical properties of the PBF denote over-bank and near-bank accretion of fine sediments under conditions of low current velocity and reduced runoff[7,14]. The early and late Holocene rejuvenation, incision and terrace formation imply increased stream energy and reduced sediment load[7,14].

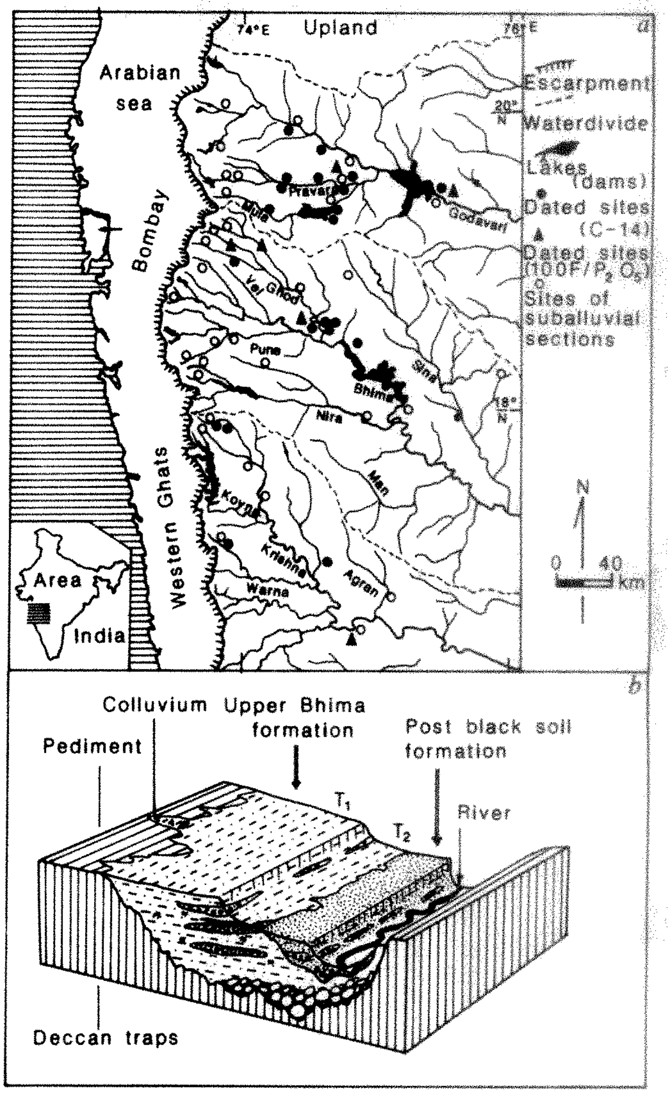

Fig. 1 Map of the area investigated (*a*); generalized block diagram showing the two-alluvial-fill and two-terrace combination in valley alluvium (*b*), T_1 = terrace 1 and T_2 = terrace 2 (not to scale).

The tentative periods of incision and deposition recognized for the uplands rivers, are consistent with the regional climatic changes of the late Quaternary, as evidenced from micro-fossil, oxygen isotope and sediment studies of deep sea cores and surface sediments from the northern Arabian sea[2,3,16,17], and from geoarchaeological studies in western India[8-10]. The late Pleistocene aggradational phase coincided with the weakening of the south-west monsoon, particularly during last glacial maximum (~18,000 yr BP) and reversal of this pattern during early Holocene increased runoff and the rivers incised. Comparison of the late Quaternary behaviour of these east flowing rivers with the sea-level changes along the west coast of India, reveals an inverse correlation between the two[18]. During low sea-level, the sea receded by >200 km and increased the continentality and aridity in the upland region, leading to aggradation (Fig. 3*a*). In contrast, high sea-level reduced the continentality and increased runoff, and the rivers incised vigorously[18]

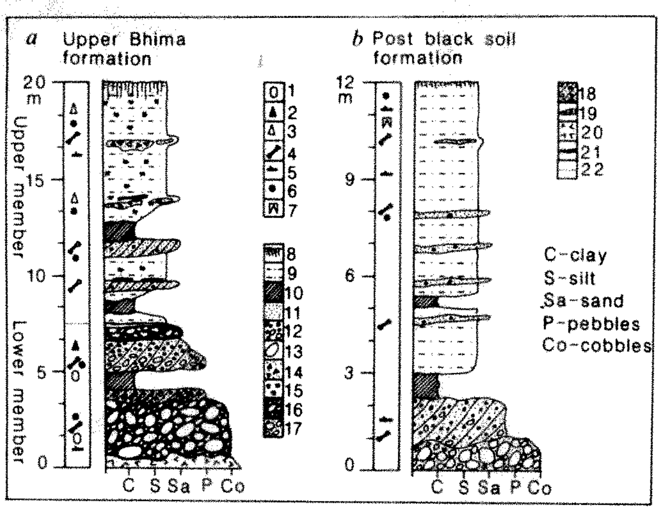

Fig. 2 Composite lithosections for the UBF (*a*) and PBF (*b*); 1, Acheuljan, 2, Middle Palaeolithic, 3, Upper and Epi-palaeolithic artefacts, 4, fossil bones, 5, fossil wood, 6, shells, 7, Chalcolithic artefacts; 8, soil, 9, sandy-silt, 10, fissured clays, 11, sand, 12, pebbly gravel 13, pebbly-cobbly gravel 14, colluvial gravel, 15, calcrete, 16, indurated gravel, 17, unconsolidated gravel, 18, cross-bedding, 19, lenses, 20, basalt, 21, bedded calcrete, 22, erosional surface.

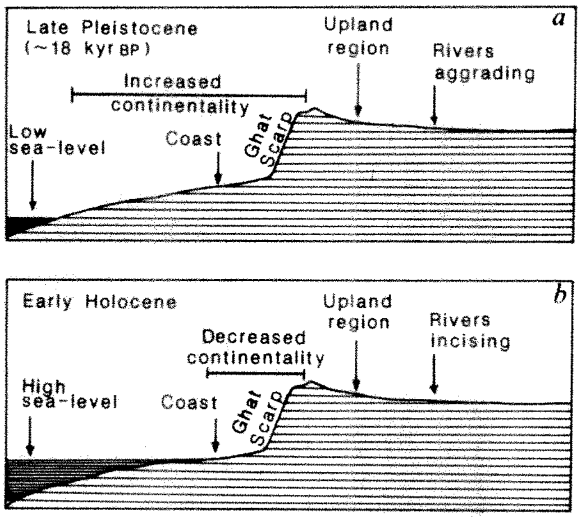

Fig. 3 Relation between the sea-level changes along the west coast of India and the nature of fluvial regimes in the upland region of northwestern Deccan between ~>30,000 and 4,500 yr BP (not to scale).

(Fig. 3*b*). Aggradation, thus, occurred during low sea-level and intense incision followed the early Holocene rapid rise in the sea-level (Fig. 3). The late Holocene changes in climate were comparatively minor and therefore do not correlate with the sea-level changes along the west coast of India.

Aridity in the study area is also evident from the thick (2-8 m) end-Pleistocene colluvial deposits in the foothills of many valleys[14]. A drier climate during the late Holocene is shown by pedocalcic soils occurring between the Chalcolithic and the early historical deposits and by changes in the dietary habits of Chalcolithic inhabitants from cultivated cereals to animal food[19]. The downfall of the Chalcolithic cultures close to one millennium BC has also been attributed to severe droughts in the western and central India[19]. Archaeological evidence in the form of cultural growth and the economic development of an early agricultural community, suggest amelioration in climate between 2,200 and 1,500 yr BP[7].

The changes in the fluvial deposition and downcutting and the environments deduced for the upland region of northwestern Deccan are similar to those of the Son and Belan in north-central India[4,5], the Nile[20], the Amazon[21] and northern Chile[22]; and coincide with global changes in climate in the intertropical zone[1,4].

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Identification of an aphid sex pheromone

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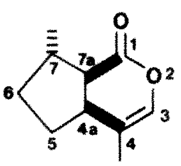
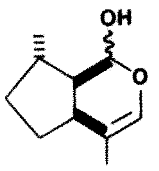
Although aphids reproduce asexually on their host plants during the summer, many species migrate to a winter host where sexual reproduction occurs. Males of certain species locate mates by means of a sex attractant pheromone released from the hind legs of the females[1-4]. Here we report the identification of such a pheromone from the vetch aphid *Megoura viciae*. Extracts of the excised legs of sexual females were analysed by gas chromatography-coupled single cell recording techniques (GC-SCR)[5] and by gas chromatography-coupled mass spectrometry (GC-MS). A tentative identification by MS analysis of the two active components as specific isomers of the monoterpenoids nepetalactone (I), a known cat attractant, and nepetalactol (II) was confirmed by comparison with authenticated compounds isolated or synthesized from natural products. Although neither compound was attractive to males when presented alone in the laboratory bioassay, a mixture of the two components produced a response equal to that elicited by the female leg extract. Thus the sex pheromone of *M. viciae* comprises a synergistic mixture of the nepetalactone I and the nepetalactol II. The identification of aphid sex pheromones will provide a further means of investigating and manipulating the behaviour of these ubiquitous pests.

Perception of the sex pheromone in aphids is thought to be associated with the secondary rhinaria on the antennae of males[4]. With electrophysiological recording techniques in which single-cell preparations are used to monitor the effluent from high-resolution gas chromatography (GC-SCR)[5] we have identified the sex pheromone of an aphid. Viviparae of *Megoura* *viciae* were maintained on bean plants, *Vicia faba*, at 15 °C in a 12 h day/night light regime in order to produce sexual forms. The oviparous females obtained were anaesthetized with ether and the hind legs removed and extracted with pentane. This extract was behaviourally active in a laboratory bioassay (Table 1). The bioassay was done in a simple choice chamber consisting of a 15 cm Petri dish, in the base of which were two holes covered with a layer of muslin. Under these holes were placed either the treatment, applied to a filter paper square, or an empty pot as a control. Ten male *M. viciae* were placed in the dish and numbers of aphids positioned over each hole (the 'test area') were recorded once a minute for 10 minutes. During the test, the dish was placed in a box, illuminated only from above, which was aired with an electric fan between tests. Female leg extract ($10\ \mu l$) was used at a concentration equivalent to 1 leg per μl of solvent. Pheromone components were tested at a similar concentration, $10\ \mu l$ being applied when tested alone, and $5\ \mu l$ of each when tested together.

Single-cell recordings showed that olfactory cells associated with the secondary rhinaria of males responded to the female leg extract (Fig. 1). GC-SCR, using these recordings to monitor the column effluent, demonstrated the presence of two electrophysiologically active components. GC-coupled mass spectrometry (GC-MS) (70 eV, 200 °C on a VG 70-250 mass spectrometer with an OV-101 bonded phase fused silica capillary column) showed that their relative molecular masses (M_r) were 168 (compound X) and 166 (compound Y). The fragmentation of compound Y was similar to that of nepetalactone, which has three known diastereoisomers[6]. These were isolated from catmint plants, *Nepeta cataria* and *N. mussinii*, by HPLC (50 × 1 cm column, $10\ \mu$ silica, Partisil Magnum 9 10/50, 5% (v/v) ether/hexane, monitoring at 208 nm) and structures were confirmed by ^{13}C and ^1H nuclear magnetic resonance (NMR) spectroscopy. The three diastereoisomers were well resolved on chromatography: (4a*S*, 7*S*, 7a*R*)-nepetalactone (I) from *N. cataria* had the same GC retention time as compound Y and the mass spectrum[6], when run under the same conditions, was identical to that from the aphid, m/z 166 (M^+, 61%), 151 (7), 138 (19), 123 (70), 109 (41), 95 (59), 81 (81), 69 (100), 67 (58), 55(35), 41 (74), 39 (53). In addition, the peak from compound Y in the aphid extract was enhanced by co-injection with the plant-derived nepetalactone I on the OV-101 and on a bonded Carbowax 20-M fused silica capillary column. The (4a*S*, 7*S*, 7a*R*)-nepetalactone was active electrophysiologically (Fig. 1), but gave only a weak response in the behavioural bioassay (Table 1). The enantiomer (4a*R*, 7*R*, 7a*S*)-nepetalactone was prepared from (*R*)-pulegone by a route based on Wolinsky's work[7] but showed no electrophysiological activity (Fig. 1). It was concluded that compound Y was (4a*S*, 7*S*, 7a*R*)-nepetalactone (I) but that for behavioural activity, compound X was required.



I



II

The mass spectrum for compound X, m/z 168 (M^+, 45%), 135 (52), 97 (57), 84 (70), 81 (72), 71 (77), 67 (61), 58 (58), 55 (52), 43 (60), 41 (100), 39 (52), did not closely resemble any published spectrum. However, iridodial has M_r 168 and gives, by McLafferty rearrangement, an abundant ion fragment at m/z 58. This compound was obtained from the devil's coach horse, *Staphylinus* (*Ocypus*) *olens*[8,9], but chromatographed before compound X and gave a very different mass spectrum. It was considered likely that compound X was a reduction product of the second component Y and the conversion of (4a*S*, 7*S*, 7a*R*)-nepetalactone into compound X was attempted. This was achieved by reduction with diisobutylaluminium hydride. The product gave a single peak on GC which was enhanced when co-injected with the aphid extract on both the OV-101 and Carbowax 20-M capillary columns and gave a mass spectrum which was similar to that obtained for the aphid compound X. The nepetalactol structure (II) was established for the reduction product by ^{13}C NMR: peaks at 93.9 p.p.m. ($-OCHOH$), 133.5 and 113.1 ($C{=}CH{-}O$) and 7 others upfield, and by ^1H NMR: characteristic peaks at 6.01 p.p.m. ($={CH}{-}O$), 4.85 ($O{-}CH{-}O$) and 2.82 (OH), with 5 Hz couplings from the 4.85 peak to both the OH peak and an octet at 1.64 (H-7a). The absolute stereochemistry of the parent compound, vis (4a*S*, 7*S*, 7a*R*), was retained. Although reduction to the lactol (II) created a new asymmetric centre at carbon-1, only one diastereoisomer was obtained. The 5 Hz coupling between Hs 1 and 7a in the NMR spectrum was not, without further study, sufficient to determine unequivocally the absolute stereochemistry at C-1. The lactol structure was claimed as a rearrangement product of iridodial but was not fully characterized[8,10].

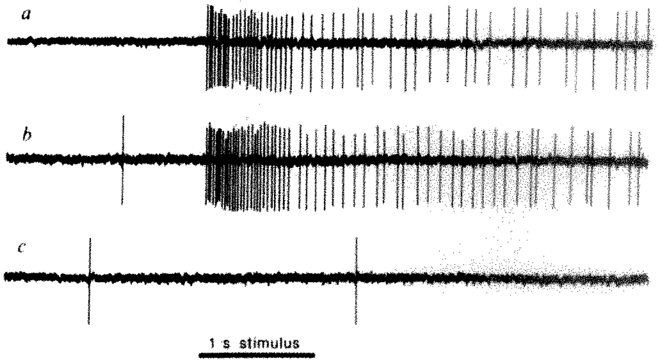


Fig. 1 Responses of a male *M. viciae* olfactory cell to stimulation with ~20 leg equivalents of *a*, female leg extract; *b*, nepetalactone I; *c*, enantiomer of nepetalactone I.

The (4a*S*, 7*S*, 7a*R*)-nepetalactol was electrophysiologically active but showed no behavioural activity when tested alone in the laboratory bioassay. However, when applied together with the (4a*S*, 7*S*, 7a*R*)-nepetalactone, male response was equal to that elicited by a female leg extract containing similar amounts of each component, ~0.5–1 ng per leg (Table 1). Thus the sex pheromone of the aphid *Megoura viciae* comprises the nepetalactone I and the nepetalactol II. The nature of the sex pheromones of other aphids is now under investigation: initial studies have shown that both the black bean aphid, *Aphis fabae* Scopoli, and the pea aphid, *Acyrthosiphon pisum* (Harris), have olfactory receptors for these two compounds. The existence of intra- and interspecific responses to aphid sex pheromones[1,2,4] suggests a lack of specificity that may prove advantageous to their use for aphid control.

The presence of nepetalactone in certain plants is unlikely to be directly associated with the composition of the aphid sex pheromone and may be the result of an economy of biosynthetic routes in the plant and animal kingdoms. Compounds closely

Table 1 Behavioural bioassay with male *M. viciae*

Treatment	No. of replicates	Mean aphid score Treated	Control	*P*
Leg extract	12	5.7 ± 0.38	0.08 ± 0.08	<0.001
Nepetalactone I	4	1.75 ± 0.25	0.5 ± 0.28	NS
Nepetalactol II	4	0.66 ± 0.66	0.66 ± 0.33	NS
I + II	12	5.75 ± 0.41	0	<0.001
No treatment	4	0.5 ± 0.5	0.5 ± 0.29	NS

Aphid score, maximum no. of aphids observed in test area; NS, not significant at probability level 0.05.

related to both the nepetalactone I and the nepetalactol II have been shown to influence the behaviour of certain aphid predators[11] and it is possible that the sex pheromone may act as a kairomone in attracting predatory species. However, although the nepetalactone I is also known to be a powerful attractant for cats and other Felidae[12], a behavioural link in this case would be hard to conceive.

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An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*

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Blooms of the toxic dinoflagellate *Gonyaulax tamarensis* (synonyms *Protogonyaulax tamarensis*[1] and *Alexandrium tamarense*[2]) cause outbreaks of paralytic shellfish poisoning (PSP) in coastal waters throughout the world. In the Gulf of Maine, episodes occur between April and November, a seasonality due in part to life-cycle alternations between motile, vegetative cells and resting cysts which overwinter in bottom sediments[3,4]. Newly formed cysts have a mandatory 2–6 month dormancy period during which germination is not possible[5], but once mature, the resting state will continue if temperatures are unfavourable[5] or oxygen is unavailable[6]. We now report another factor controlling germination of cysts of *G. tamarensis* from deep coastal waters—an endogenous annual clock that can override an otherwise favourable environment for germination. Similar annual variability in germination has not been observed for cysts of this species from shallow estuaries. These results represent the first conclusive demonstration of an endogenous circannual rhythm in a marine plant. They are evolutionarily and ecologically significant because an endogenous annual clock can lead to the release of motile cells into deep and relatively invariant bottom waters at those times when temperature and light at the surface are suitable for growth. In shallow waters where seasonal variability is large and extends to bottom sediments, a strategy similar to that of the seeds of terrestrial plants would be more appropriate, namely a direct coupling between germination and the external environment.

The annual rhythm was first observed in cysts from freshly collected sediment cores. Regardless of depth of burial within the sediment or overlying water depth, germination frequency of cysts from the Gulf of Maine oscillated systematically during the two-year study (Fig. 1*b*). Cysts of other species from the Gulf of Maine and of the same species from a shallow Cape Cod estuary had high germination frequencies in the same tissue culture plates and medium.

The germination rhythm was also evident in monthly subsamples from an August core stored in the dark at 2 °C (Fig. 1*c*).

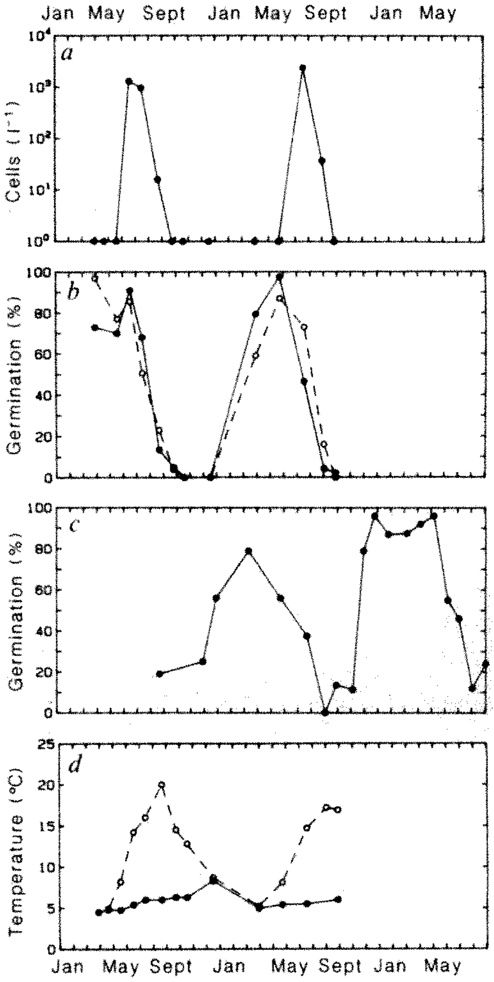

Fig. 1 Population dynamics of cysts and motile cells of *Gonyaulax tamarensis*. *a*, Average motile cell concentration in the top 30 m of the water column from continuously pumped samples at Station 29 (160 m deep, 43° N 70°19′ W) and Station 30 (60 m deep, 43°01′ N, 70°33′ W) in the Gulf of Maine. *b*, Average germination frequency of cysts isolated from (●) top 2 cm and (○) 5- and 6-cm depths in freshly collected cores from Station 29 and Station 30. *c*, Germination frequency of cysts isolated from sediment stored in the laboratory under constant conditions. Sediment from the 3–6-cm depth interval of cores from Station 29 on 17 August 1984. *d*, Bottom (●) and surface (○) water temperature at Station 29. **Methods.** Cores were extruded on board ship, the outer circumference discarded, and the central portion of each depth interval stored in the dark at the temperature of the bottom water during transit to the laboratory. Within 48 h, the samples were sonicated, sieved[17], and the 20–80-μm fraction resuspended in germination medium. To maintain constant germination conditions, all cyst suspensions and germination medium used sea water from the same two polyethylene carboys of Sargasso Sea water, filtered through a 0.2-μm filter and diluted 10% with distilled, deionized water. This was considered more consistent in composition and more conducive to germination than artificial sea water. Germination medium was 'f/2' of Guillard and Ryther[18]. Each data point in *b* and *c* represents the germination success of at least 48 cysts that were isolated and placed individually in separate wells of a 96-well tissue culture plate in 130 μl of medium. Plates were taped to minimize evaporation and incubated on a 14:10 h light to dark photoperiod at 15 °C and 150 μE m^{-2} s^{-1} (cool-white fluorescent light) for one month. Excystment was successful if the germling cell was able to emerge completely from the cyst wall. The germination data in *c* were obtained using small aliquots removed from the central portion of a large sediment sample collected in August 1984 from Station 29. Subsamples were processed and cysts isolated and incubated as described above. The bulk mud sample was stored in the dark at 2 °C.

Thus the reduced germination success from August to December is not due to the primary dormancy of immature cysts; maturation would be evidenced by an increasing trend in germination frequency through time, remaining constant and high thereafter. Persistence of the germination rhythm for two years under constant storage conditions indicates endogenous control.

Annual cycles have long been recognized in plants and animals[7], but many of the rhythms occur in response to external cues and disappear with storage in constant conditions. True endogenous control of annual rhythms is most easily demonstrated in animals[8] and is difficult to document in plants[9]. The germination of some plant seeds can vary over an annual cycle, in that seeds can be alternately responsive and unresponsive to the same germination conditions[10,11], but this rhythm typically disappears with storage in constant conditions. The phenomenon is thus termed secondary dormancy—the environmentally mediated re-induction of a state of deep dormancy. The germination rhythm in *G. tamarensis* cysts isolated from monthly core samples (Fig. 1*b*) could be attributed to secondary dormancy as the 'storage' environment could not be controlled. However, persistence of the rhythm for two years under constant conditions in the laboratory (Fig. 1*c*) is consistent with endogenous regulation.

Suggestions of annual rhythms are found in three studies of *G. tamarensis*. Yentsch and Mague[12] report evidence suggestive of an annual cycle in maximum growth rate and cell yield. The data are not conclusive, however, as they were compiled from the control cultures of a series of experiments conducted in different growth chambers by several investigators. Dale *et al.*[13] and Fukuyo *et al.*[14] could not germinate *G. tamarensis* cysts from Gulf of Maine and Ofunato Bay sediments during the fall months. Both studies attributed the lack of germination to immature cysts, but it is also possible that both used cysts in the unresponsive phase of their annual cycle. Interestingly, germination of the cysts of a closely related strain (or species[1,2]) of *Gonyaulax* did not follow a rhythmic pattern during the Ofunato Bay study[14]. Similarly, during this study and others[15], we observed high germination frequencies for cysts of *G. tamarensis* from a shallow estuary throughout the year. This implies that the annual rhythm is maintained only in certain populations or strains of this organism.

Control of cyst germination by an endogenous clock would be most useful in deep coastal waters where bottom temperatures are relatively invariant and photoperiodic cues non-existent. Without endogenous regulation, cyst germination would occur throughout the year, because bottom temperatures in the Gulf of Maine remain within a permissive 4–6 °C range (Fig. 1*d*). Instead, we observe a cyst germination rhythm with the same seaonality as the motile cell blooms (Fig. 1*a*). If further studies verify that there is no similar timing mechanism in shallow water strains of *G. tamarensis*, an environmental separation of genotypes is implied. This would not be surprising as a genetic basis for circadian rhythms has been reported in the fruit fly *Drosophila*[16]. Shallow water sediments experience considerable environmental variability from year to year, so inflexible endogenous control of germination might be a disadvantage compared to a strategy whereby germination is directly controlled by the environment. Shallow water populations of *G. tamarensis* may thus be comparable to terrestrial plants whose seeds are responsive only to the external environment for the breaking of dormancy.

More work is needed to explain the observed annual rhythm and to understand differences between strains or populations. If endogenous annual rhythms are found in the germination patterns of other cyst-forming dinoflagellates or in the growth or metabolism of phytoplankton in general, such information would have profound implications with respect to bloom dynamics and the temporal succession of algal species.

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Transient cells of the developing mammalian telencephalon are peptide-immunoreactive neurons

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In the development of the mammalian telencephalon, the genesis of neurons destined for the various layers of the cerebral cortex is preceded by the generation of a population of cells that comes to reside in the subplate and marginal zones[1] (see ref. 2 for nomenclature). In the cat, these cells are present in large numbers during development, when their location is correlated with the arrival and accumulation of ingrowing axonal systems[3–6] and with synapses[7–12]. However, as the brain matures, the cells disappear and the white matter and layer 1 of the adult emerge[1,13,14]. Their disappearance occurs in concert with the invasion of the cortical plate by the axonal systems and with the elimination of the synapses from the subplate[1,4,7,9,12]. Here we report that the subplate cells have properties typical of mature neurons. They have the ultrastructural appearance of neurons and receive synaptic contacts. They also have long projections and are immunoreactive for MAP2 (microtubule associated protein 2). Further, subpopulations are immunoreactive for one of several neuropeptides. These observations suggest that during the fetal and early postnatal development of the mammalian telencephalon the subplate cells function as neurons in synaptic circuitry that disappears by adulthood.

We identified the subplate and marginal zone cells unambigously by their birthdate. These cells undergo their final round of cell division and migrate away from the ventricular zone between embryonic day 24 (E24) and E30 (gestation is 65 days in the cat)[1]. In contrast, cells of the cortical layers 2–6 are not generated until after E30[15]. Accordingly, intrauterine injections of ^{3}H-thymidine (NEN, 50–80 Ci mmol^{-1}) in sterile physiological saline were made between E26 and E28 in anaesthetized fetuses[1]. Ten fetuses were then allowed to develop to later fetal or early postnatal (P) ages: E50 (2 animals), E54 (1), E56 (3), E58 (1) and P7 (3). An additional ten animals at comparable stages of development were examined without prior thymidine labelling. Immunohistochemical and/or retrograde labelling studies were performed and then sections labelled with ^{3}H-

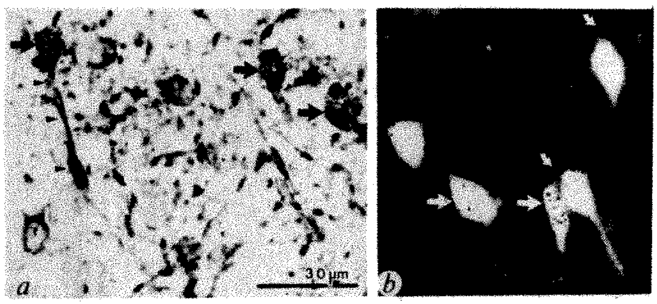

Fig. 1 Subplate cells (*a*) stained with antisera against MAP2, and (*b*) labelled retrogradely following an injection of TRITC into the contralateral hemisphere. Subplate cells were identified by their distinct birthdates with ^{3}H-thymidine labelling at E27. Cells shown in the photographs of each panel were located deep within the future white matter. In *a*, the brain was prepared for MAP2 immunohistochemistry at E56 by perfusing with 4% paraformaldehyde in 0.1 M sodium phosphate buffer followed by immersion-fixation in a mixture of periodate-lysine-paraformaldehyde[34]. Cryostat sections (10 μm) were cut and incubated in 1:1000 anti-MAP2[16] or normal rabbit serum. The distribution of specific antibody binding was visualized using peroxidase immunohistochemistry (Vector Labs). Reacted sections were then processed for autoradiography. Arrows, MAP2-immunoreactive cells that are also heavily labelled with ^{3}H-thymidine, as indicated by the accumulation of silver grains over each nucleus. (Immunoblot analysis indicates that MAP2 is present at E56. See also ref. 30.) A common morphological feature of these cells is the presence of a large dendritic process (arrowheads) directed towards the ventricular surface of the telencephalon (downwards in this figure). In *b*, subplate cells (^{3}H-thymidine-labelled cells indicated by arrows) are shown to have a long projecting process (probably an axon), as they can be retrogradely labelled following an injection of TRITC deep into the subplate of the opposite hemisphere. Note that processes can extend from the soma either towards the pial surface (curved arrows) and/or towards the ventricular surface (arrowheads). TRITC (Sigma) was injected as a 10% solution in dimethylsulfoxide; multiple injections (0.1 μl) into the contralateral hemisphere were followed by a 48-h survival period. Animals were perfused at P7 (4% paraformaldehyde), and brain sections (80 μm) made on a vibratome were processed for autoradiography and viewed with a fluorescence microscope.

thymidine were autoradiographed to confirm the identity of the early generated cells.

The subplate cells have characteristics of neurons such as immunoreactivity for the neuron-specific protein microtubule-associated protein 2 (MAP2)[16,17]: every ^{3}H-thymidine-tagged subplate cell was MAP2 immunoreactive (Fig. 1*a*, arrows). Many of these cells possess a distinctive process often directed towards the ventricular surface (Fig. 1*a*, arrowheads); this and other processes emanating from the soma are frequently beaded. MAP2 is thought to be dendrite-specific at immunohistochemically detectable levels both in adults and during development[16,17], so these processes are probably dendrites.

In addition to dendrites, the subplate cells also have long projecting processes, probably axons. This was demonstrated by retrograde labelling experiments in which multiple injections of the fluorescent tracer tetramethylrhodamine isothiocyanate (TRITC) were made deep into the subplate of one hemisphere in eight neonates. Figure 1*b* shows several TRITC-labelled cells in the subplate of the opposite hemisphere that are similar in morphology to the ^{3}H-thymidine-tagged cells immunoreactive for MAP2. These were found in both the injected as well as the opposite hemisphere. This labelling technique also occasionally revealed processes directed toward the pial surface (Fig. 1*b*, curved arrows; see also Fig. 3*a*). Moreover, some of the TRITC-labelled cells can be autoradiographically labelled by ^{3}H-thymidine injection made at E27 (Fig. 1*b*, thick arrows), thereby identifying them as subplate cells.

The subplate cells have the ultrastructural appearance of neurons. Figure 2 shows an electron micrograph of a subplate cell retrogradely labelled with horseradish peroxidase (HRP). Electron-dense inclusion bodies characteristic of retrogradely transported HRP are located within the distinctive processes (Fig. 2*a*, thin arrows), which at higher magnification (Fig. 2*b*) have the morphological characteristics of dendrites including ribosomes and the presence of synaptic contacts. Thus, a variety of different critera indicate that the subplate cells are neurons.

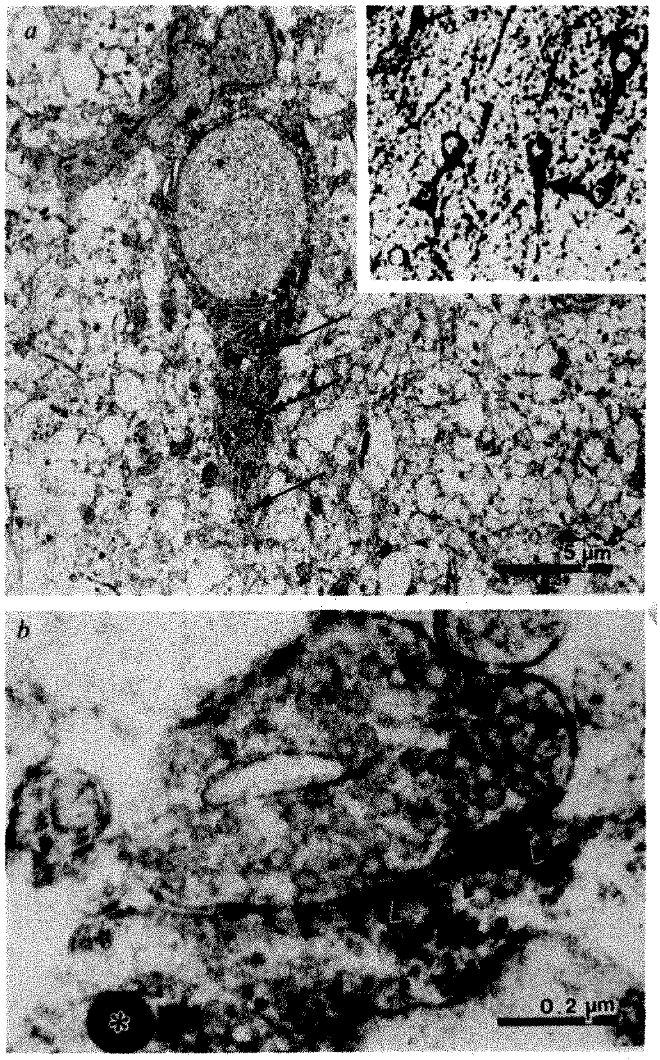

Fig. 2 Ultrastructural evidence showing that subplate cells can receive synapses. Subplate cells were retrogradely labelled in a newborn cat by injecting 40% HRP, 1% lysolecithin in sterile saline, deep into the subplate. The animal was reanaesthesized 24 h later, perfused with mixed aldehydes, and the brain processed for EM-HRP histochemistry using DAB-nickel-cobalt as the chromogen[35]. In *a*, the inset shows a light micrograph of retrogradely labelled subplate cells (arrow). The pial surface is towards the top of the page. One of the cells in the inset is shown in the electron micrograph. The arrows indicate electron dense, HRP inclusion bodies typical of retrogradely labelled cells. *b*, Higher magnification view of a portion of the distinctive process of a subplate cell identified by the presence of an HRP inclusion body (asterisk). Note the synapse (arrowheads) and the presence of ribosomes that identify the postsynaptic profile as a dendrite.

To characterize these neurons further, their immunoreactivities for several neuropeptides were examined, an approach motivated in part by recent observations that the few neurons embedded in the white matter of adult mammalian neocortex are frequently immunoreactive for neuropeptides[18-22]. As previous ^{3}H-thymidine birthdating studies have shown that <10% of the subplate neurons persist into adulthood, where they are

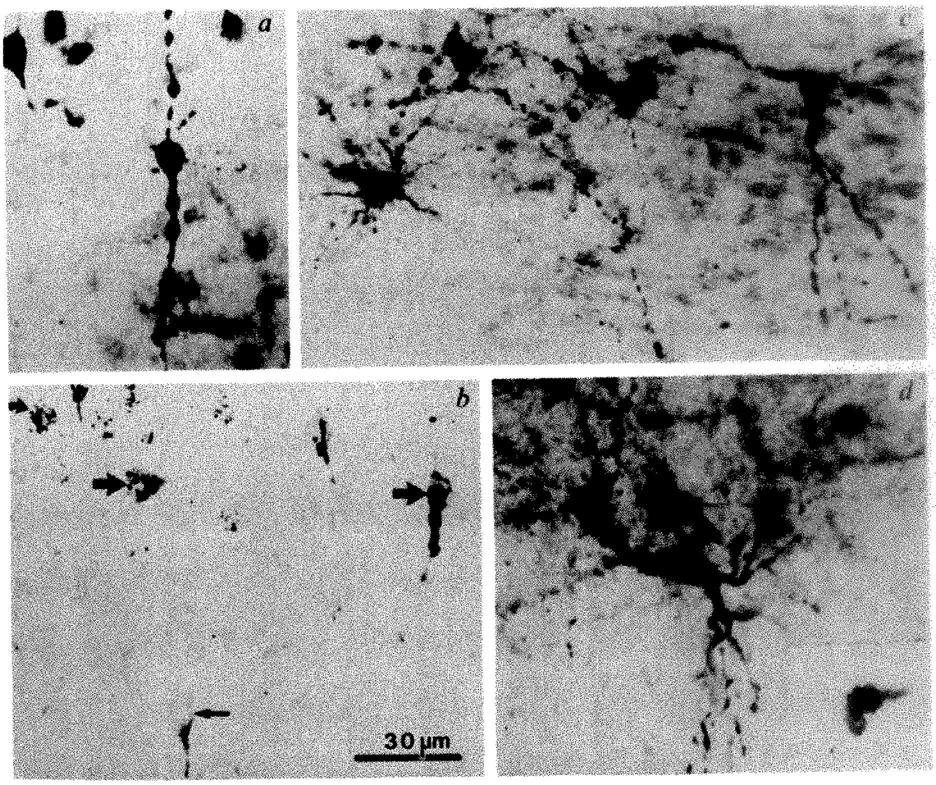

Fig. 3 Subplate cells immunoreactive for the neuropeptide (*a*, *b*) SRIF, (*c*) NPY and (*d*) CCK. In each case, fetuses received an injection of ^{3}H-thymidine at E27 to label the subplate cells and at later ages, the brains were prepared for immunohistochemistry as described for Fig. 1. All antisera were used at a dilution of 1:1,000 (SRIF and CCK, Immunonuclear Corporation; NPY, gift from Dr C. Evans, Stanford University). The pattern of peptide immunostaining was specific as it was not present in normal rabbit serum controls (1:1,000) and could be blocked routinely by incubating 50 nM of the appropriate peptide (Peninsula Labs) simultaneously with diluted antisera. The true TRQRYamide carboxy terminus, against which the NPY antiserum was raised, completely blocked staining at 15 nM whereas the same concentration of two similar amidated carboxy termini (YPMRFamide, TRPRYamide) did not. Staining for each antiserum was unaffected by simultaneous incubation with the other two peptides. Sections were cut on a vibratome at 80 µm (*c*, *d*) or cut on a cryostat at 10 µm for subsequent autoradiography (*b*, *d* inset). In *a* and *c*, the morphological appearance at E54 of cells immunoreactive for either SRIF or NPY is often similar to that of the subplate cells shown in Fig. 1. Note the many beaded processes in the 80 µm sections, some of which can be followed for several millimeters both away from and up into the cortical plate. In *b*, some of the cells immunoreactive for SRIF are indeed subplate cells since they can be tagged with ^{3}H-thymidine given at E27 (thick arrows). Note that not all cells generated on E27 are SRIF-immunoreactive (curved arrow), nor are all SRIF-positive cells born at E27 (thin arrow). Subplate cells and marginal zone (future layer 1) cells have the same birthdates[1], and both populations can be immunoreactive for CCK. An example of a CCK-immunostained marginal zone cell at age P7 is shown in *d*. The inset shows a double-labelled marginal zone cells to show that the birthdate of some CCK-immunostained marginal zone cells is E27. Pial surface is always towards the top of the figure.

located in the white matter[1], it seemed likely that the peptide immunoreactive cells of the adult white matter are the residuum of the transient subplate cell population that have survived into adult life. When sections from developing brains between E50 and P7 were tested for neuropeptide immunoreactivity, specific immunostaining of cells in the telencephalon was found for neuropeotide Y (NPY), somatostatin (SRIF) and cholecystokinin (CCK). Figure 3 shows examples of cells in the subplate immunoreactive for SRIF (Fig. 3*a*) and NPY (Fig. 3*c*) at E54. Immunostaining for SRIF and NPY could first be detected by E50, but that for CCK could not be observed until around E60. Also, the majority of CCK-immunoreactive cells are found in the marginal zone (Fig. 3*d* and Fig. 4), whereas all of the NPY- and SRIF-immunoreactive cells are located in the subplate or deepest layers of the cortical plate.

Some if not all of these peptide-immunoreactive cells belong to the early generated population of subplate and marginal zone cells, for they can be tagged with ^{3}H-thymidine at E27. For example, the autoradiograph of Fig. 3*b* shows that some of the subplate cells with silver grains over their nuclei are also immunoreactive for SRIF at E54. In addition, double-labelled cells (Fig. 3*b*, large arrows) are intermixed with neurons born at the same time but not SRIF-immunoreactive (Fig. 3*b*, curved arrow), indicating that subplate neurons born at any particular time are heterogeneous with respect to transmitter phenotype. Figure 3*b* also shows that some of the SRIF immunoreactive cells are not labelled with ^{3}H-thymidine (thin arrows). Nevertheless, many of these immunostained but not thymidine-labelled cells are probably subplate neurons as only a small fraction of the entire subplate population can be labelled by a single ^{3}H-thymidine injection[23]. Similar conclusions can be drawn for the NPY- and CCK-immunostained populations (Fig. 3*d*, inset).

To examine whether there is a relationship between peptide immunoreactivity and location within the telencephalon three adjacent sections from a P7 cat brain were immunostained with one of the three antibodies against NPY, SRIF or CCK and the distribution of immunoreactive cells recorded. Figure 4 shows the results of this comparative analysis. Counts of the number of labelled cells per section indicate that about 75% of all NPY-immunoreactive cells are embedded within the white matter/lower subplate, whereas ~70% of all of the SRIF-immunoreactive cells are found more superficially in a region located immediately beneath the cortical plate: the upper subplate (see ref. 1 for definitions of the upper and lower subplate). Finally, 65% of all CCK-immunoreactive cells reside within the marginal zone, where they comprise the only peptide-immunoreactive population observed there. Thus cells immunoreactive for a given peptide tend to reside within a specific tangential domain of the telencephalon.

The results of this study show that the subplate cells, identified by ^{3}H-thymidine birthdating, are neurons. First, all are found to be immunoreactive for the neuron- and dendrite-specific marker MAP2. Second, these cells can project locally within the subplate and can extend long processes to the opposite hemisphere. Third, they resemble neurons ultrastructurally and receive synapses. Fourth, subpopulations are immunoreactive for at least one of three neuropeptides: CCK, SRIF and NPY.

The morphology of the early generated cells studied here is remarkably similar to that of Golgi-impregnated cells in previous studies of developing cortex. The marginal zone cells immunoreactive for CCK resemble the transient Cajal–Retzius cells[14,24–27]. The morphology of many subplate neurons is similar to that of the Martinotti cell or interstitial neuron[13,26,28,29]. We suggest that most if not all of these cell types belong to the early-generated, transient population that we have defined with the various double-label techniques of the present study. Although conclusive proof for this suggestion requires ^{3}H-thymidine identification of Golgi stained material, it is supported

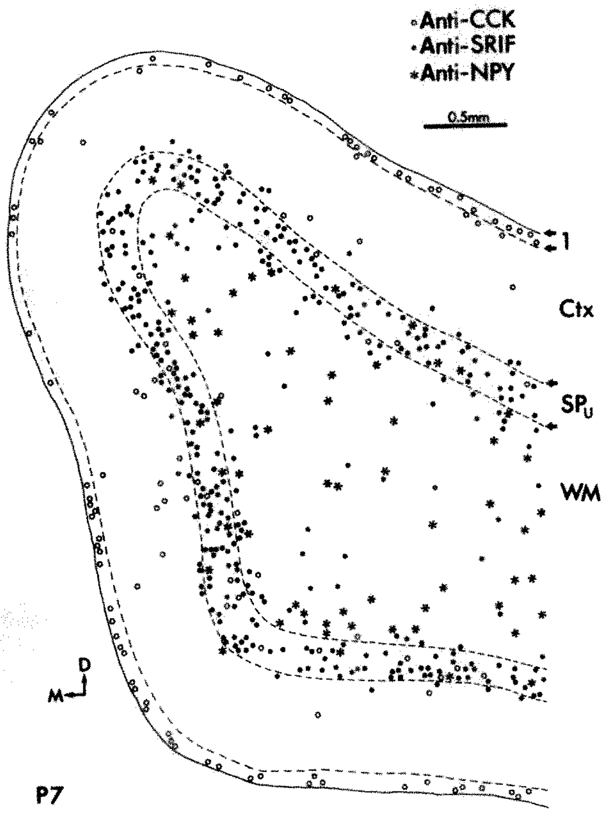

Fig. 4 The peptide immunoreactivity of subplate cells varies with their tangential position. Three adjacent 80-μm-thick coronal sections through the visual cortex of a P7 cat were immunostained with one of the antisera against CCK, NPY or SRIF. Each section was drawn with a camera lucida attachment and the location within the telencephalon of all labelled cells with a given peptide immunoreactivity was marked. The three drawings were then superimposed. Symbols identify cells immunoreactive for a given peptide: open circles, CCK; closed circles, SRIF; asterisks, NPY. Note the exclusive presence of CCK immunostained cells in layer 1, the clustering of SRIF immunostained cells in the upper subplate (SP_U), and the NPY immunostained cells deep in the white matter (WM). Ctx, cortex; M, medial; D, dorsal.

by two recent observations. Firstly, MAP2-immunoreactive cells thought to be Cajal–Retzius cells have been found in the marginal zone of mice embryos[30] and secondly in the telencephalon of human fetuses, some cells located in the subplate are also immunoreactive for SRIF[31].

The subplate cells are neurons that receive synaptic inputs during development. The most likely sources of the presynaptic inputs are the ingrowing thalamocortical and corticocortical axons, which accumulate in the subplate when the subplate cells are present[3-6,32,33]. Thus, the subplate cells may serve as the temporary targets of ingrowing afferent systems. If so, these cellular elements could represent part of an early and transient neural circuit that precedes the establishment of adult cortical connectivity. In addition, the distinct tangential domains occupied by cells of a given neuropeptide immunoreactivity suggest that different groups of subplate cells could serve distinct functions during the development of the mammalian cerebral cortex.

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A glucagon fragment is responsible for the inhibition of the liver Ca^{2+} pump by glucagon

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Glucagon specifically inhibits the Ca^{2+} pump in liver plasma membranes independently of adenylate cyclase activation[1]. However, this inhibition is only observed at high concentrations of glucagon ($K_i = 0.7\ \mu M$). Moreover, in the presence of bacitracin, an inhibitor of glucagon degradation[2], the Ca^{2+} pump is no longer sensitive to glucagon[3]. These findings suggest that a fragment of glucagon might be the true effector of the liver Ca^{2+} pump. Pairs of basic amino acids are recognized as potential cleavage sites in post-translational processing of peptide hormones[4-6]. The glucagon molecule includes a dibasic doublet (Arg 17–Arg 18). Therefore, we have examined the action of glucagon(19–29) on the liver Ca^{2+} pump. This peptide was obtained from glucagon by tryptic cleavage and separated by reverse-phase high-performance liquid chromatography. We found that glucagon(19–29), which is totally ineffective in activating adenylate cyclase, inhibited both the Ca^{2+}-activated and Mg^{2+}-dependent ATPase activity ((Ca^{2+}-Mg^{2+}) ATPase) and Ca^{2+} transport in liver plasma membranes with an efficiency 1,000-fold higher than that of glucagon. Glucagon(1–21) was completely inactive; glucagon(18–29) and glucagon(22–29) acted only as partial agonists of glucagon(19–29). These results indicate that glucagon(19–29), obtained by proteolytic cleavage of glucagon, is likely to be the active peptide involved in the inhibition of the liver Ca^{2+} pump. We suggest that glucagon may be a precursor of at least one biologically active peptide.

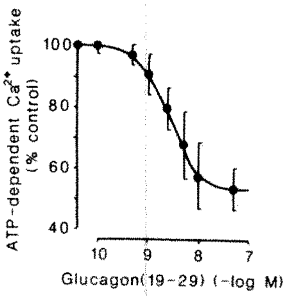

Fig. 1 Inhibition by glucagon(19-29) of the ATP-dependent Ca^{2+} uptake by liver plasma membrane vesicles. Results are the mean of five different experiments, done with five different plasma membrane vesicle preparations; bars, ±s.e.m.

Methods. Plasma membrane vesicles were prepared from liver of female albino Wistar rats (100-120 g body weight) according to Prpic *et al.*[7], using a Percoll gradient. Glucagon(19-29) was prepared as described in Fig. 3 legend, from crystalline pancreatic glucagon provided by Novo Laboratories (Paris, Copenhagen). Ca^{2+} uptake was assayed in inside-out plasma membrane vesicles as previously described[1]. Preincubation at 37 °C for 5 min of plasma membrane vesicles (120-320 μg protein) with glucagon(19-29) (0-50 nM) diluted in 50 mM Tris-HCl, *p*H 8, 0.01% bovine serum albumin (BSA) and 0.87 μM lactose, allowed the peptide to interact with intravesicular sites of inside-out vesicles because of the leakiness of vesicles. After 5 min, the incubation was run in 200 μl of assay medium containing 50 mM Tris HCl, *p*H 8, 0.01% BSA, 400 μM EGTA, 393 μM CaCl$_2$ (0.1 μM free Ca^{2+}), 2 μCi ml^{-1} of ^{45}Ca, 180 mM sucrose, 1 μM ruthenium red, 20 mM sodium azide, 4 mM Tris-oxalate, 10 mM MgCl$_2$, with either no ATP added or 10 mM ATP. MgCl$_2$ in the assay medium is used to seal vesicles[1,28]. After 15 min, 150 μl samples were filtered under vacuum on Millipore filters (HAWP 0.45 μM) that had been soaked in 0.25 M KCl for 1 h. The filters were washed three times with 5 ml iced 250 mM sucrose, 40 mM NaCl, dried, and counted in 10 ml of Beckman Ready Solv. The ATP-dependent Ca^{2+} uptake was determined from the difference in radioactivity bound to the filter in the presence and absence of ATP. Uptake is expressed as a percentage of control ATP-dependent Ca^{2+} uptake (6.1 ± 1.7 nmol Ca^{2+} per mg protein per 15 min).

The ATP-dependent Ca^{2+} uptake was studied in liver plasma membrane vesicles prepared according to Prpic *et al.*[7]. Addition of glucagon(19-29) (0-50 nM) to the assay medium resulted in a dose-dependent inhibition of the ATP-dependent Ca^{2+} uptake (Fig. 1). A maximal inhibition of 40-60% was attained with 10 nM glucagon(19-29), half-maximal inhibition occurring at 3 ± 0.3 nM glucagon(19-29).

From kinetic studies[1] and reconstitution experiments using the purified enzyme (in preparation), we have concluded that the high-affinity (Ca^{2+}-Mg^{2+}) ATPase is the enzyme responsible for Ca^{2+} transport. Accordingly, (Ca^{2+}-Mg^{2+}) ATPase activity was assayed in purified liver plasma membranes prepared according to Neville[8] up to step 11. This preparation was chosen because it has been used for the characterization of numerous membrane enzymes and receptors[9-13]. Glucagon(19-29) induced a dose-dependent inhibition of the (Ca^{2+}-Mg^{2+}) ATPase activity (Fig. 2). Maximal inhibition was 15-20% at 10 nM glucagon(19-29), and half-maximal inhibition occurred at 0.75 ± 0.25 nM glucagon(19-29). Inhibition was due to a decrease in maximal velocity of the reaction with no change in the apparent affinity of the enzyme for calcium (data not shown). Also, (Ca^{2+}-Mg^{2+}) ATPase was assayed in parallel with Ca^{2+} uptake in liver plasma membrane vesicles prepared according to Prpic *et al.*[7]. In these preparations, inhibition of (Ca^{2+}-Mg^{2+}) ATPase by glucagon(19-29) reached 15-20% at 10 nM glucagon(19-29), half-maximal inhibition being observed for 1 ± 0.25 nM glucagon(19-29) (data not shown).

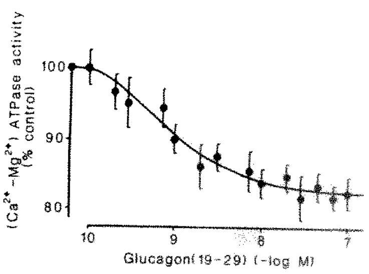

Fig. 2 Inhibition of (Ca^{2+}-Mg^{2+}) ATPase activity in purified liver plasma membranes by glucagon(19-29). Results are the mean of ten different experiments done with five different membrane preparations; bars, ±s.e.m.

Methods. Purified plasma membranes were prepared from liver of female albino Wistar rats (100-120 g body weight) according to Neville[8] up to step 11. Plasma membranes (4-8 μg protein) were preincubated at 4 °C with glucagon(19-29) (0-0.1 μM) diluted in 50 mM Tris-HCl, *p*H 8, 0.01% BSA and 0.87 μM lactose. After 10 min, (Ca^{2+}-Mg^{2+}) ATPase activity was assayed as previously described[1]. The assay medium contained in a final volume of 200 μl, 0.25 mM ATP, 50 mM Tris-HCl, *p*H 8, 400 μM EGTA and 393 μM CaCl$_2$ (0.1 μM free Ca^{2+}). After 10 min incubation at 30 °C, aliquots were assayed for P$_i$ by colorimetric determination[29]. Basal activity was estimated for each point by omitting CaCl$_2$ from the assay medium and (Ca^{2+}-Mg^{2+}) ATPase activity, calculated by subtracting values obtained in the presence of chelator alone from those obtained with chelator plus CaCl$_2$, is expressed as a percentage of control (Ca^{2+}-Mg^{2+}) ATPase activity (1.2 ± 0.2 nmol P$_i$ per mg protein per 10 min).

Native glucagon caused 25 and 50% maximal inhibition of liver (Ca^{2+}-Mg^{2+}) ATPase and Ca^{2+} transport respectively, but its apparent affinity for the Ca^{2+} pump is in the micromolar range (ref. 1, Table 1). Therefore, it appears that glucagon(19-29) is 1,000-fold more efficient than native glucagon in inhibiting the liver Ca^{2+} pump. To assess the specificity of the action of glucagon(19-29), we investigated the action of three other glucagon fragments: glucagon(18-29), glucagon(22-29) and glucagon(1-21). Glucagon(18-29) was tenfold less potent than glucagon(19-29) in inhibiting (Ca^{2+}-Mg^{2+})ATPase (Table 1 and Fig. 3). Glucagon(22-29) produced only a 5-15% maximal inhibition of (Ca^{2+}-Mg^{2+}) ATPase, with a low potency (1 μM), similar to that of native glucagon (Table 1). The amino-terminal (1-21) fragment of glucagon had no effect on (Ca^{2+}-Mg^{2+}) ATPase (Table 1) nor did it antagonize inhibition induced by either native glucagon or glucagon(19-29).

Table 1 Effects of various glucagon fragments on (Ca^{2+}-Mg^{2+}) ATPase activity in liver plasma membranes

	Glucagon(19-29)	Glucagon(18-29)	Glucagon	Glucagon(22-29)	Glucagon(1-21)
K_i (nM)	0.75 ± 0.5	7.5 ± 2.5	700 ± 300	1,000 ± 300	10,000
Inhibition (% maximal)	20 ± 5	15 ± 5	25 ± 10	10 ± 5	—

The (Ca^{2+}-Mg^{2+}) ATPase activity was measured as in Fig. 2 legend, in the presence of 0-10 μM of either glucagon(19-29), glucagon(18-29), glucagon, glucagon(22-29) or glucagon(1-21). A 10-min preincubation at 4 °C of plasma membranes (3-8 μg protein) with the peptide was used, followed by a 10-min incubation at 30 °C, under the conditions described in the legend to Fig. 2. Results are the mean of four different experiments using three different plasma membrane preparations, and are expressed as a percentage of control activity (1.3 ± 0.2 μmol Pi per mg protein per 10 min). Sources: glucagon(1-21), a gift from Novo Laboratories; glucagon(22-29) from Peninsula Laboratories; glucagon(19-29) and glucagon(18-29), prepared from glucagon as described in Fig. 3 legend.

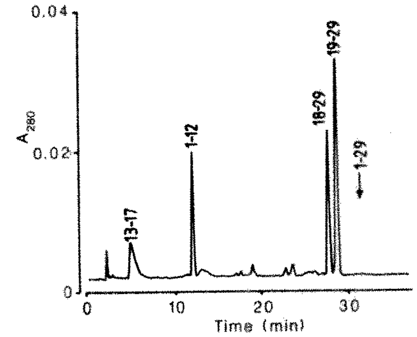

Fig. 3 Preparation of glucagon(19–29) and glucagon(18–29) by HPLC. Arrow, elution volume of intact glucagon(1–29).

Methods. Crystalline porcine pancreatic glucagon was digested for 1 h at 37 °C in 50 mM Tris HCl, 1 mM $CaCl_2$, *p*H 7.5, with tosylphenylethyl-chloromethyl-ketone (TPCK)-treated trypsin (Sigma) at a 1:100 (w/w) enzyme/substrate ratio. After boiling for 3 min, the peptide mixture was separated by reverse-phase high-performance liquid chromatography (RP-HPLC) on a Waters apparatus equipped with a μBondapak C_{18} column run at 1.5 ml min^{-1} in 0.1% trifluoroacetic acid. The peptides were eluted with a linear gradient (0–50% in 50 min) of acetonitrile containing 0.05% ethylacetate[30]. Peptides were monitored by UV absorption at 214 and 280 nm, UV spectra were obtained on-line from the HPLC peaks using a Hewlett-Packard model 8450A diode array spectrophotometer. The purity of the peptide fragments was assessed by comparing their UV spectra (not shown) with theoretical spectra obtained with a computer databank (D.B. and M.D., manuscript in preparation) and by amino-acid analysis performed with RP-HPLC of OPA derivatives[31]. The results obtained (theoretical values in parentheses) were: glucagon(19–29): Ala 1.1 (1), Asp 2.2 (2), Glu 2.4 (2), Phe 1.3 (1), Leu 1.2 (1), Met 0.8 (1), Arg 0.14 (0), Thr 1.1 (1), Val 0.9 (1); Glucagon(18–29): Ala 1.3 (1), Asp 2 (2), Glu 2.2 (2), Phe 1.1 (1), Leu 0.9 (1), Met 0.8 (1), Arg 1.3 (1), Thr 1.3 (1), Val 0.9 (1). The presence of intact tryptophan in both peptides was assessed by their UV spectra (not shown).

The integral glucagon molecule is required for full activation of adenylate cyclase. In particular, neither glucagon(1–21)[14] nor glucagon(22–29)[15] can stimulate adenylate cyclase activity. We did not observe any effect of glucagon(19–29) (0–0.1 mM) on adenylate cyclase activity (not shown). This is in keeping with our previous observation that inhibition of the Ca^{2+} pump by glucagon is independent of cyclic AMP production[1].

Our results clearly indicate that nanomolar concentrations of glucagon(19–29) specifically inhibit the Ca^{2+} pump in liver plasma membranes. Furthermore, the present data show that the amino-terminal residues in glucagon(19–29) are essential. Indeed, the addition of an arginine residue (peptide (18–29)) or the suppression of residues 19–21 (peptide (22–29)) resulted in a dramatic loss of potency. Thus, glucagon(19–29) appears to be the molecular form of glucagon responsible for the inhibition of the calcium pump in the liver plasma membrane. The metabolic consequences of the action of glucagon(19–29) on liver cells, in particular its effect on the glycogenolytic pathway, remain to be elucidated, as does the nature of the specific receptor which recognizes this peptide.

Two hypotheses can be raised concerning the physiological precursor of glucagon(19–29). Firstly, glucagon(19–29) could be released during the processing of proglucagon as are other glucagon-related peptides[16-20]. Alternatively, glucagon(19–29) could be generated locally upon interaction of the circulating glucagon with hepatocytes. The existence of a trypsin-like protease associated with liver plasma membranes has been demonstrated[21]. Conversion of peptide hormones by the target tissue has already been described for growth hormone[22], proinsulin[23], angiotensin 1[24], and arginine vasopressin[25]. Also, cleavage of growth hormone[26] and arginine vasopressin[25] has been shown to generate new biological activities.

It was generally accepted that adenylate cyclase activation was the only enzymatic pathway regulated by glucagon. Wakelam *et al.*[27] recently reported that subnanomolar concentrations of glucagon cause a breakdown of inositol phospholipids independently of cyclic AMP production suggesting that glucagon may also regulate phospholipase C. Our findings provide a new mechanism by which glucagon may regulate liver cell metabolism through an enzymatic pathway distinct from adenylate cyclase or phospholipase C, and point out the possible role of glucagon as a precursor of peptides with new biological activities.

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Enhanced translation of chimaeric messenger RNAs containing a plant viral untranslated leader sequence

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Eukaryotic messenger RNAs are translated with unequal efficiencies *in vivo*[1,2] and *in vitro*[3-5] and the molecular basis of this phenomenon is not understood. As an approach to understanding the role of the 5′ untranslated leader sequence in regulating mRNA translational efficiency, chimaeric mRNAs have been generated by joining a heterologous leader to complementary DNA (cDNA) sequences, followed by *in vitro* transcription using SP6 RNA polymerase and *in vitro* protein synthesis. We used the untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV

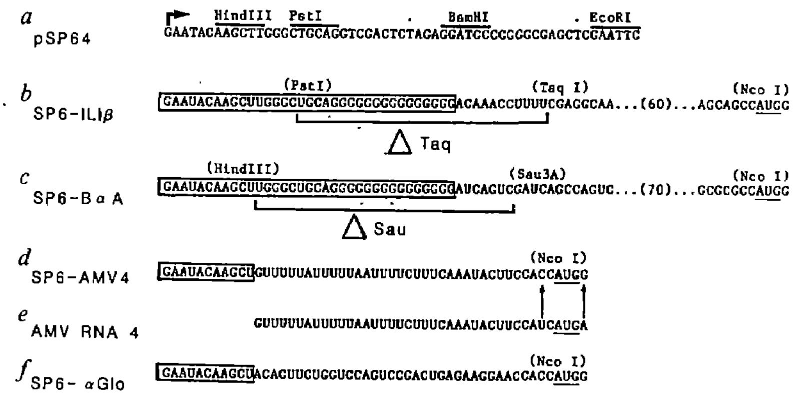
a
pSP64
HindIII PstI BamHI EcoRI
GAATACAAGCTTGGGCTGCAGGTCGACTCTAGAGGATCCCCGGGCGAGCTCGAATTC
b
SP6-IL1β
(PstI) (Taq I) (Nco I)
GAAUACAAGCUUGGGCUGCAGGGGGGGGGGGGGGGACAAACCUUUUCGAGGCAA...(60)...AGCAGCCAUGG
Δ Taq
c
SP6-BαA
(HindIII) (Sau3A) (Nco I)
GAAUACAAGCUUGGGCUGCAGGGGGGGGGGGGGGGAUCAGUCGAUCAGCCAGUC...(70)...GCGCGCCAUGG
Δ Sau
d
SP6-AMV4
(Nco I)
GAAUACAAGCUGUUUUUAUUUUUAAUUUUCUUUCAAAUACUUCCACCAUGG
e
AMV RNA 4
GUUUUUAUUUUUAAUUUUCUUUCAAAUACUUCCAUCAUGA
f
SP6-αGlo
(Nco I)
GAAUACAAGCUACACUUCUGGUCCAGUCCGACUGAGAAGGAACCACCAUGG

Fig. 1 Nucleotide sequences of the 5′ untranslated leaders. *a*, As a reference, the transcriptional start site (arrow) and the polycloning region of plasmid SP64 are shown; *b*, *c*, corresponding regions of SP6-IL1β and SP6-BαA. Square brackets, nucleotides deleted during construction of Δ*Taq*-IL1β and Δ*Sau*-BαA; numbers in parentheses, nucleotides in the untranslated leader of IL-1β or BαA which are present in the construct but not shown in the figure. *d*, Corresponding regions of chimaeric AMV mRNAs; and *e* of native AMV RNA 4 strain 425[26]. Upward arrows, nucleotides changed during construction of the SP6-AMV4 untranslated leader (*d*). *f*, Corresponding regions of SP6-rabbit α-globin. In all the constructs, underlines indicate the initiation codon for protein synthesis and boxed nucleotides indicate vector sequences upstream of the 5′ end of the cDNA inserts which are transcribed by SP6 polymerase. Identical nucleotides in each sequence have been aligned vertically. To avoid ambiguities in the data which might be attributed to codon context surrounding the initiator AUG[11,27,28], the leader-coding sequence junction of all DNA constructs has been standardized upon the recognition sequence for the restriction enzyme *Nco*I (CCATGG, where ATG is the initiation codon). Barley α-amylase[8], human interleukin 1β[9] and α-globin[29] cDNAs have naturally occurring *Nco*I sites separating the 5′ untranslated leader sequence and the protein coding region (*b*, *c*, *f*).

Methods. SP6-IL1β and SP6-BαA were constructed by inserting the human interleukin 1β cDNA[9] and the type A barley α-amylase cDNA[8] into the *Pst*I-*Bam*HI or *Pst*I sites, respectively, of pSP64 (Promega Biotec). To construct Δ*Taq*-IL1β, a 30-base-pair *Pst*I-*Taq*I fragment was removed[30] from SP6-IL1β. A 31-base-pair *Hin*dIII-*Sau* 3A fragment was removed from SP6-BαA to create Δ*Sau*-BαA. An oligodeoxyribonucleotide representing the sequence of the AMV RNA 4 leader (modified to create an *Nco*I site at the 3′ terminus) was synthesized as complementary strands which were annealed and ligated into the *Hin*dIII-*Nco*I sites of SP6-IL1β or SP6-BαA to create AMV-IL1β or AMV-BαA. An oligodeoxyribonucleotide representing the rabbit α-globin untranslated leader[29] was similarly synthesized and inserted into SP6-BαA to create αGlo-BαA.

RNA 4), a well-translated, highly competitive message[5,6], to replace the leader sequence of barley α-amylase (BαA) and human interleukin 1β (IL-1β) cDNAs. Deletion of transcribed vector sequences and replacement of the native untranslated region with the AMV RNA 4 leader can result in as much as a 35-fold increase in mRNA translational efficiency; moreover, the translational efficiency of the chimaeric mRNAs containing the AMV RNA 4 leader is at least as great as that of virion RNA 4. The results suggest that the chimaeric AMV-mRNAs have either a higher relative affinity or a diminished requirement for a limiting component(s) of the translational machinery; in addition, it may be feasible, through use of heterologous leader sequences, to increase expression of engineered genes or cDNAs without changing the antigenic or biological properties of the encoded protein.

Because of the correlation between absence of stable 5′ secondary structure and enhanced translational efficiency of AMV RNA 4[7], we decided to test the role of the AMV RNA 4 untranslated leader in facilitating translation by transferring the sequence to the 5′ terminus of other mRNAs. The SP6-IL1β and SP6-BαA subclones contain a 5′ G_{15} sequence added during cloning[8,9]; therefore, the SP6 RNA transcripts contain 20 nucleotides of 5′ SP6 vector sequence followed by 15 guanosine residues preceding the cDNA sequence (Fig. 1*b*, *c*). The 5′ poly(G) sequence was deleted from the IL-1β cDNA by excising a 30-base-pair fragment ending in a *Taq* I site just inside the untranslated leader (Fig. 1*b*). The Δ*Taq*-IL1β mRNA was translated with greater efficiency in both protein-synthesizing systems than the SP6-IL1β mRNA containing the poly(G) sequence (Fig. 2*a*, *b*). In a third construct, the AMV RNA 4 untranslated leader was substituted for the IL-1β leader by first excising a *Hin*dIII-*Nco*I fragment from SP6-IL1β DNA, and then inserting a 37-base-pair AMV RNA 4 leader oligonucleotide (Fig. 1*d*). The 5′ nucleotide sequence of the chimaeric SP6 mRNAs containing the AMV RNA 4 leader is shown in Fig. 1*d*. The translational efficiency of the chimaeric AMV-IL1β mRNA increased 2–2.5-fold over the Δ*Taq*-IL1β mRNA and the total increase in translational efficiency resulting from removal of the homopolymer tail and native IL-1β leader, followed by substitution of the AMV RNA 4 untranslated leader sequence, was ~6–7-fold (Fig. 2*a*, *b*).

Qualitatively similar results were obtained using the BαA cDNA, although the SP6-BαA mRNA stimulated protein synthesis *in vitro* minimally, and deletion of the 5′ poly(G) sequence had a more dramatic effect on translational efficiency than observed with the IL-1β mRNA (Fig. 2*c*, *d*). The G_{15} sequence was removed from the SP6-BαA subclone by excising a 31-base-pair fragment ending at a *Sau*3A site immediately within the untranslated leader sequence (Fig. 1*c*), and as shown in Fig. 2*c*, *d*, the corresponding Δ*Sau*-BαA mRNA translated well in both reticulocyte lysate and wheat-germ extract. Substitution of the AMV RNA 4 leader for the BαA leader was performed as for the IL-1β construct. The chimaeric AMV-BαA mRNA was translated with 2–3-fold greater efficiency than Δ*Sau*-BαA, giving a total increase, in comparison with SP6-BαA, of ~10-fold in the wheat-germ extract and 35-fold in the reticulocyte lysate (Fig. 2*c*, *d*). Data from control experiments demonstrated that all mRNAs were stable during the translation experiments and that unequal capping efficiences did not form the basis of the differential translational efficiencies. Analysis of the translation products by SDS-polyacrylamide gel electrophoresis verified that the qualitative patterns of proteins translated from the chimaeric or non-chimaeric mRNA were similar and that the composition of the untranslated leader sequence had a notable effect upon the quantity of protein synthesized (data not shown).

A detailed analysis of codon context requirements for optimal translation of an mRNA in transfected COS cells[10,11] found that rat preproinsulin mRNA containing an adenosine (A) residue at position −3 relative to the initiation codon was translated with greater efficiency than the same mRNA with a guanosine (G) at position −3. To test the possibility that the increased translational efficiency of chimaeric mRNAs was due only to changing the G at −3 present in IL-1β and BαA to the A found in AMV RNA 4, an oligodeoxyribonucleotide representing the untranslated leader of rabbit α-globin (Fig. 1*f*) was used to replace the BαA leader. The α-globin leader is characterized by an A at position −3 and the nucleotide sequence is the same length as the AMV RNA 4 leader (Fig. 1*d*, *f*); moreover, the α-globin untranslated leader ends at an *Nco*I restriction site which is located within the optimal codon consensus sequence and putative ribosome binding site[10,11] (Fig. 1*f*). If the preferred codon context found in the chimaeric AMV-mRNAs by itself resulted in increased translational efficiency, then use of the rabbit α-globin leader sequence, with its ideal context, would

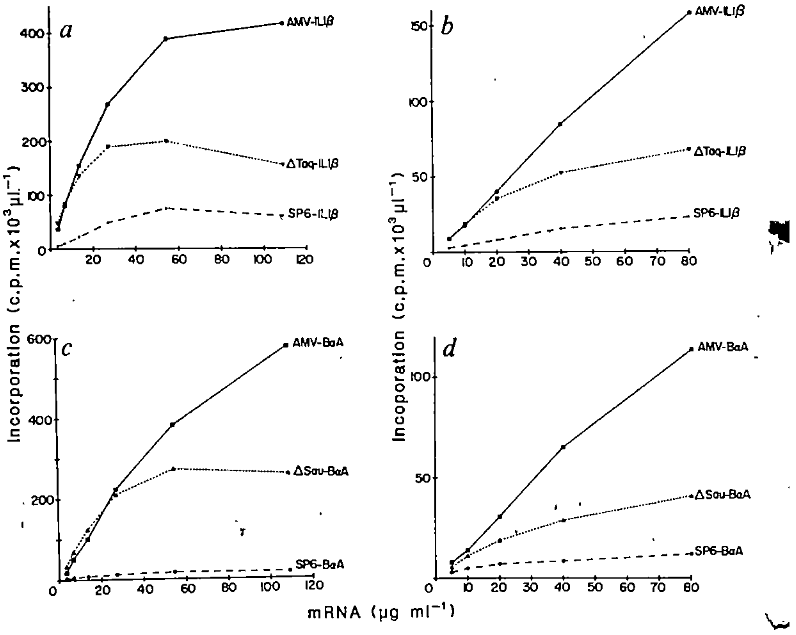

Fig. 2 Analysis of the translational efficiency of SP6 mRNAs and chimaeric AMV-mRNAs *in vitro*. Incorporation of ^{35}S-methionine is shown as a function of RNA concentration for IL-1β or BαA mRNAs translated in either the rabbit reticulocyte lysate (*a*, *c*) or wheat-germ extract (*b*, *d*).

Methods. Capped SP6 mRNAs were transcribed from linearized DNAs[31], and low-molecular-weight materials were removed by passing the DNase-treated transcription reaction through two successive Sephadex G-50 spun columns[30]. The eluate was then extracted twice with phenol/chloroform, once with ether, and nucleic acids were precipitated with ethanol from 2.5 M ammonium acetate. Translations were performed essentially according to the manufacturer's recommendations. Wheat-germ translations were prepared using 5 μl of nuclease-treated wheat-germ extract (Amersham) in a total volume of 10 μl containing 105 mM potassium acetate, 2 mCi ml^{-1} ^{35}S-methionine, and the reaction mixture was incubated for 30 min at 25 °C. Reticulocyte translations were performed using 7 μl nuclease-treated rabbit reticulocyte lysate (Promega Biotec) in a total reaction volume of 11 μl containing 2 mCi ml^{-1} ^{35}S-methionine, and the reactions were incubated for 15 min at 30 °C. In all examples, the salt conditions were optimized for the RNAs being translated, and incorporation of isotope was linear with respect to time during the incubation period. Duplicate 1-μl aliquots were removed for quantitation of hot-acid-precipitable radioactivity. The counts per min in the water control translations (without added mRNA) have been subtracted before plotting (reticulocyte lysate: 7,220 c.p.m. μl^{-1}; wheat-germ extract: 2,580 c.p.m. μl^{-1}).

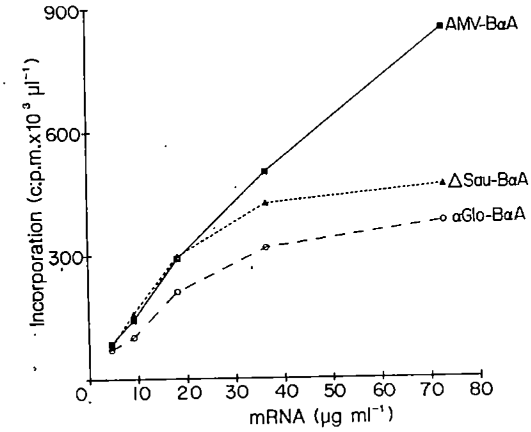

Fig. 3 The enhanced translational efficiency of the chimaeric AMV-mRNAs is a result of a feature of the leader other than the purine at position −3 and codon context effects.

Methods. Barley α-amylase mRNAs containing either the AMV-, Δ*Sau*-, or α-Glo-untranslated leaders (see Fig. 1 for details) were translated in the reticulocyte lysate as in Fig. 2. Both the AMV- and α-globin untranslated leaders have perfect consensus sequences surrounding the initiation codon[10,11,27] whereas the Δ*Sau* leader has a guanosine residue at position −3. (Control without mRNA: 14,500 c.p.m.)

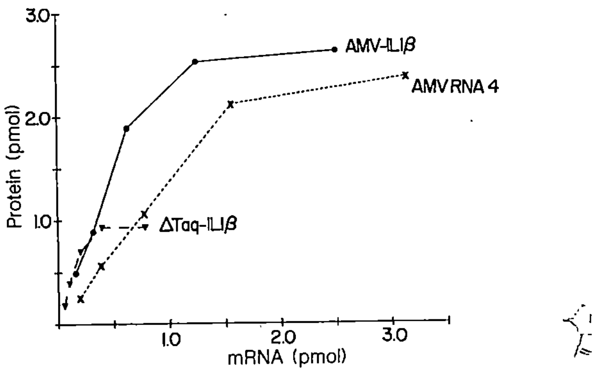

Fig. 4 The translational efficiency of the chimaeric AMV-mRNAs is similar to that of virion AMV RNA 4. Translation of AMV-IL1β and Δ*Taq*-IL1β mRNAs in the reticulocyte lysate was compared to that of an SP6 transcript of the AMV RNA 4 cDNA. This SP6-AMV 4 mRNA translates with efficiency equal to that of virion RNA 4[17]. To normalize the data for differences in the length of the mRNAs and for the methionine content of the proteins, quantities have been expressed in moles.

Methods. Plasmid pSP65A4, yielding full-length transcripts of AMV RNA4[17] was linearized with *Sma*I, and capped messenger RNAs were transcribed and translated as in Fig. 2.

also be expected to increase translation of chimaeric mRNAs. However, the translational efficiency of the chimaeric αGlo-BαA was significantly less than that of AMV-BαA and similar to Δ*Sau*-BαA (Fig. 3), showing that the increased translational efficiency of the chimaeric AMV-RNAs (Fig. 2) is related to features of the leader other than the purine at position −3 and codon context effects.

When translated at concentrations ⩾20 μg ml^{-1}, the chimaeric AMV-mRNAs stimulate further incorporation of ^{35}S-methionine, whereas protein synthesis directed by the non-chimaeric mRNA reaches a plateau. These results suggest that a component required for efficient translation of the non-chimaeric mRNAs becomes limiting at high mRNA concentrations. In contrast to poliovirus infection[12], plant RNA viruses do not cause a 'shut-off' of host protein synthesis[13]; however, coat protein mRNAs of many viruses possess properties providing for enhanced translation[14–16], and coat protein is synthesized in massive quantities. Loesch-Fries *et al.*[17] have reported that mRNA transcribed from plasmid pSP654A4, containing the complete AMV RNA 4 cDNA, translates with the same efficiency as virion mRNA. The translational efficiencies of AMV-IL1β, Δ*Taq*-IL1β, and AMV RNA 4 (from plasmid pSP65A4) were compared in the reticulocyte system (Fig. 4). The translational efficiency of the chimaeric AMV-IL1β was slightly greater than that of AMV RNA 4, but that of Δ*Taq*-IL1β, although similar to AMV-IL1β at low mRNA concentrations, failed to increase stimulation of protein synthesis at high concentrations. The translational properties of the chimaeric AMV-mRNAs seem to mimic those of the viral coat protein mRNAs through a characteristic elevated mRNA saturation concentration and highly efficient, competitive protein synthesis[5,6,18].

These studies support several conclusions regarding the role of the 5′ untranslated leader sequence. First, the untranslated leader contains information which regulates both translational efficiency and the mRNA saturation point during translation *in vitro*. Second, the presence of the poly(G) region in the untranslated leader inhibits translation of the mRNA in a manner suggested by other work[19]. Comparison of the translational efficiencies of SP6-IL1β and SP6-BαA (and L.G. and S.A.J. unpublished data) suggest that the poly(G) region is not inherently inhibitory, but may inhibit translation through interactions with other sequences within the mRNA. Third, an adenosine residue at position -3 relative to the AUG initiation codon is not necessarily correlated with optimal translational efficiency[3,4,11] (Fig. 3). Regulatory regions in addition to codon context are therefore important in determining mRNA translational efficiency. Fourth, the AMV RNA 4 untranslated leader sequence affects the translational characteristics of the mRNA even when located 11 nucleotides downstream of the 5′ cap structure (Fig. 1). This result suggests that the enhanced translational efficiency of the chimaeric AMV-mRNAs is not signalled by a unique nucleotide sequence located directly at the 5′ mRNA terminus, but may relate to the secondary structure of the untranslated leader and the potential for formation of stable base pairs within the leader and between the leader and the 5′ proximal coding sequence[20,21].

Messenger RNA secondary structure has been suggested repeatedly as an important factor in determining mRNA translational efficiency[6,20-22]; however, little is known about how cells discriminate among mRNAs for translation. AMV RNA 4, which is principally unstructured at the 5′ terminus[7] may not require the 'unwinding' activity of the cap-binding protein complex[21,23]; moreover, the mRNA is indeed translated in extracts prepared from poliovirus-infected HeLa cells where the high-molecular-weight component of the cap-binding complex is inactivated by proteolytic cleavage[24]. Secondary structure analyses indicate that the single-stranded nature of the AMV RNA 4 leader is maintained in the chimaeric AMV-mRNA constructs[25]. Our data are consistent with the hypothesis[7,21] that mRNAs containing the AMV RNA 4 untranslated leader have a diminished requirement for a factor such as cap-binding protein complex, and that this diminished requirement is at least partially responsible for efficient, competitive translation and maximal synthesis at high mRNA concentration. The data also indicate that regulatory information carried in RNA may be transferred to heterologous sequences; this approach may also be useful for optimizing translational efficiency of transfected sequences *in vivo*, where the non-coding nature of the leader sequence may be suitable for increasing expression without altering the biological or antigenic properties of the translated protein.

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Inhibition of alloreactive cytotoxic T lymphocytes by peptides from the α_2 domain of HLA–A2

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Class I major histocompatibility complex (MHC) molecules function in the recognition of antigens by cytotoxic T lymphocytes (CTL)[1,2]. Although this biological role is firmly established and much has been learnt about their structure and polymorphic variation[3-6], little is known of the regions of class I molecules that are involved in functional interactions with components of the T-cell surface. Here we show that peptides derived from residues 98–113 of the α_2 domain of HLA–A2 specifically inhibit the recognition of target cells by many HLA–A2-specific CTL. In addition to identifying a region that is probably involved in binding the T-cell receptor these results raise the possibility that alloreactive CTL may recognize degraded fragments of class I histocompatibility antigens.

Class I MHC molecules control the recognition of membrane-associated antigens by CTL. For example virus-specific CTL recognize a particular combination of a viral antigen and a class I molecule[1,7]. This phenomenon of MHC restriction is demonstrable because of the large polymorphism of class I molecules in most mammalian species. As a consequence class I molecules are major transplantation antigens and the targets for alloreactive CTL[8,9]. Recent experiments have shown that the antigen receptors of virus-specific CTL preferentially recognize degraded fragments of viral proteins that become membrane associated[7,10]. Whether alloreactive CTL recognize intact and/or degraded class I molecules is not known.

CTL target specificity is sensitive to changes in the structure of human class I molecules (HLA-A,B,C) and can discriminate between serologically identical molecules[2,11-13]. Structural comparisons of such molecules have identified residues where substitutions affect the recognition by specific CTL. Analysis of H-2K^b

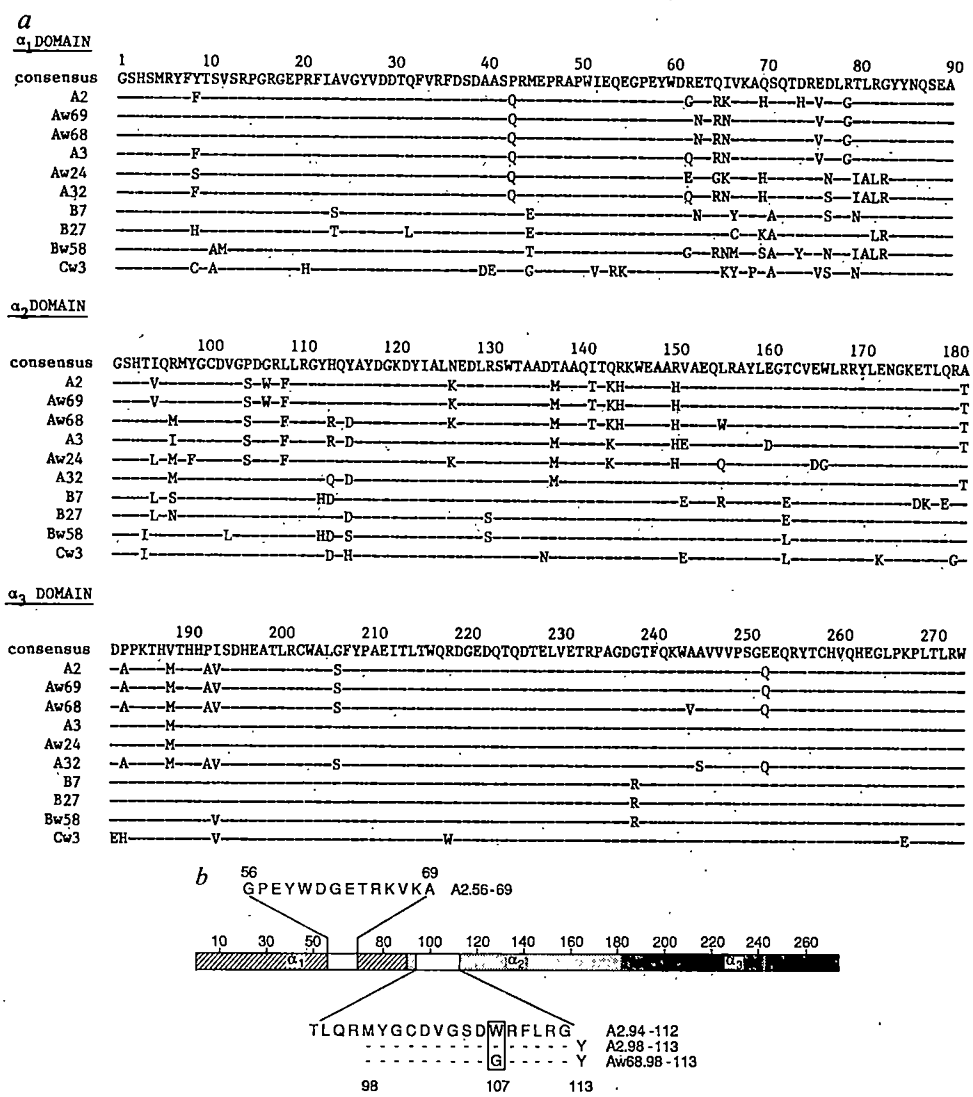
a
α1 DOMAIN
consensus GSHSMRYFYTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASPRMEPRAPWIEQEGPEYWDRETQIVKAQSQTDREDLRTLRGYYNQSEA
A2
Aw69
Aw68
A3
Aw24
A32
B7
B27
Bw58
Cw3
α2 DOMAIN
consensus GSHTIQRMYGCDVGPDGRLLRGYHQYAYDGKDYIALNEDLRSWTAADTAAQITQRKWEAARVAEQLRAYLEGTCVEWLRRYLENGKETLQRA
α3 DOMAIN
consensus DPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRW
b
56
69
GPEYWDGETRKVKA A2.56-69
α1
α2
α3
TLQRMYGCDVGSDWRFLRG A2.94-112
Y A2.98-113
G Y Aw68.98-113
98
107
113

Fig. 1 *a*, The amino-acid sequences of the three extracellular domains (α_1, α_2, α_3) of HLA-A2, Aw69 and Aw68 are compared to seven other HLA-A,B,C molecules. The heavy chain which associates with β_2-microglobulin consists of three amino-terminal extracellular domains (α_1, α_2, α_3) a transmembrane anchoring region and a short cytoplasmic domain. The three extracellular domains are thought to interact with CTL and only they are depicted. Sequence variability is concentrated in the α_1 and α_2 domains. With the exception of A32[39], Aw68 and Aw69[23], the sequences were obtained from a compilation in ref. 22 which also contains the primary references for each sequence. The consensus sequence is derived from a total of 23 HLA-A,B,C sequences by taking the most frequent residue at each position. Sequences are given in the standard one-letter code and identity with the consensus is indicated by a dash. *b*, Diagram showing the regions of the HLA-A,B,C heavy chain from which the peptides used in this study were derived. Peptides were synthesized by standard solid-phase methods[38] and their sequences are given in the standard one-letter code. The sequences of A2.98-113 and Aw68.98-113 were confirmed by automatic Edman degradation using a gas phase sequencer and shown to be >95% pure.

Table 1 Specificity of CTL tested for inhibition by peptides

CTL	Donor	Specificity of CTL	Target cell	Target molecule	Inhibition of lysis by peptide A2.98-113	A2.94-112	Aw68.98-113	A2.56-69
line AJY	AK	A2	JY	A2	+	+	–	–
line PJY	PP	A2	JY	A2	+	+	–	–
clone A20.1	AK	A2	JY	A2	+	+	–	–
clone AI.10	AK	A2	JY	A2	+	+	–	–
clone AI9.1*	AK	A2, Aw68, Aw69	JY	A2	+	+	–	–
line PWSB	PW	A2, B17	JY	A2	+	+	–	–
line PWSB	PW	A2, B17	FMB	B17	–	–	–	–
clone AL8.1	AK	Aw68, Aw69	LB	Aw68	–	–	–	ND
clone AI5.1	AK	Aw69	IDF	Aw69	–	–	–	ND
line CJY	CC	Dr6	JY	DR6	–	ND	–	–
line CJY	CC	Dr6	DAUDI	DR6	–	ND	–	–

The specificity of the CTL is based upon analysis of the pattern of killing on a panel of B lymphoblastoid cell lines and by patterns of inhibition with monoclonal antibodies. The only restriction on CTL killing are the molecules listed under 'Specificity of CTL'. ND indicates not done. CTL used were from four donors with the following HLA types: AK (A3; B7, w38; C-; DR6), PP (A1, 3; B7, w62; Cw3; DR4), CC (A2, 26; Bw50; Cw6), PW (A2; Bw46, Cw1,3). All MHC class I-specific CTL were inhibited by monoclonal antibodies against monomorphic (PA2.6, W6/32) and appropriate polymorphic (PA2.1, MA2.1, CR11-351 for A2) determinants of the class I heavy chain. No inhibition was seen with anti-β_2-microglobulin (BBM.1). The MHC class II-specific CTL were inhibited by monoclonal antibodies against monomorphic epitopes of HLA-DR (Ca.206) but not anti-class I[34].

* The observation that clone AI9.1 has specificity for both A2 and Aw68, but is only inhibited by the HLA-A2 peptide A2.98-113 and not the HLA-Aw68 peptide may result from the involvement of two regions; 98-113 and 140-160 in the CTL-target interaction. It is necessary to postulate that the interaction in the 98-113 region is stronger for A2 than Aw68 and this could be compensated by a stronger interaction with Aw68 in the 140-160 region. In this situation equivalent lysis of A2 and Aw68 targets would be associated with a more potent inhibition by peptide A2.98-113 over peptide Aw68.98-113. In this regard it should be noted that the only amino acids (threonine 142 and histidine 145), and monoclonal antibody-defined epitopes, that are specific to A2, Aw68 and Aw69 are found in the region 140-160.

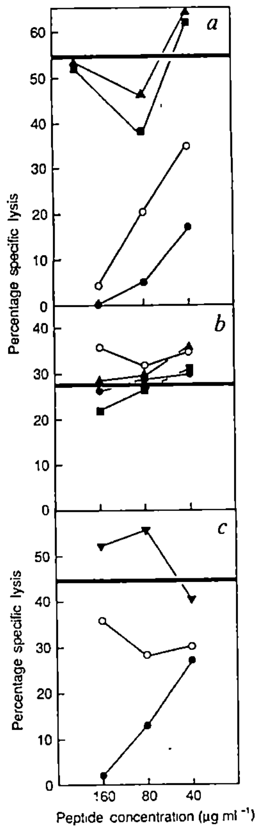

Fig. 2 *a*,*b*, Inhibition of HLA-A2 specific cytotoxic T cells (CTL) by peptides; *c*, destruction of inhibition by proteolysis. *a*,*b*, Peptide preparations were preincubated for 30 min with 1-3 × 10^3 CTL before addition of 10^3 ^{51}Cr-labelled B lymphoblastoid target cells. The peptides were present at the concentrations indicated throughout the cytotoxicity assay, which was performed as described[24,37]. In *a* the CTL is AJY, a long term $CD8^+$ cell line specific for HLA-A2 and the target cell is the B lymphoblastoid cell line JY (HLA-A2, B7). In *b* the CTL is PWSB (a bulk culture with reactivity against HLA-B17) and the target is FMB, which expresses HLA-A1,A32,B17,44. In each case the percentage specific release obtained in the absence of peptide is given by the bold horizontal line. The lower amount of specific release in panel *b* potentially made cytolysis more sensitive to inhibition. Peptides are: ●, A2.98-113; ○, A2.94-112; ▲, Aw68.98-113; ■, A2.56-69. Stocks of peptides at 1 mg ml^{-1} in phosphate-buffered saline (PBS) were diluted to give final concentrations in the assay as indicated. The final molar concentrations used in the assay at the middle dilution were 3.9×10^{-5} M for A2.98-113, 3.6×10^{-5} M for A2.94-112, 4.2×10^{-5} M for Aw68.98-113 and 4.7×10^{-5} M for A2.56-69. As a control inhibitor the monoconal antibody PA2.6, which is directed against a monomorphic determinant of HLA-A,B,C molecules, was used[37,40]. *c*, Proteolytic degradation destroys the inhibition of A2 specific CTL by HLA-A2 derived peptides. Aliquots of 1 mg ml^{-1} peptides in PBS or PBS alone were incubated for 16 h at 37 °C with various proteinases or buffer at 25 μg ml^{-1}. An additional and equivalent amount of proteinase was added after 6 h of incubation. The reactions were stopped by addition of 100 mM phenylmethylsulphonyl fluoride (PMSF) in 100% ethanol to a final concentration of 0.3 mM. These preparations were used as inhibitors of the CTL assay as described above. CTL used were an HLA-A2-specific clone designated AJ20.1 that derives from an HLA-A2-specific CTL line. The target is the JY lymphoblastoid B-cell line (HLA-A2; B7). Results shown are ●, A2.98-113 treated with buffer; ○, A2.98-113 treated with proteinase K; ▼, Aw68.98-113 treated with buffer. Incubation with trypsin and elastase had no effect on the inhibitory capacity of any peptide and chymotrypsin gave results that were similar to proteinase K. Controls included incubations with proteinases alone and with PMSF alone and these preparations did not affect the CTL assay. For each sample the degradation was monitored by HPLC on a C3 reverse phase column and was judged to be complete.

mutants in mouse[3,14] and of HLA-A2[15,16], A3[17], B7[18] and B27[19] variants in man have focused attention on residues 147-157 in the second extracellular domain of the class I heavy chain, although other substitutions can produce functional differences[20]. With this approach direct identification of the parts of the class I molecule that interact with CTL receptors is not possible, for it does not distinguish substitutions within such sites from those located in other regions that affect conformation. To address this question we have examined whether synthetic peptides derived from the HLA-A2 sequence[21] interfere with the activity of alloreactive CTL.

Sequence comparisons of HLA-A,B,C molecules show clusters of variability at the carboxy-terminal end (residues 62-83) of the α_1 domain and at the amino-terminal end (residues 94-116) of the α_2 domain (ref. 22; Fig. 1*a*). We therefore asked whether synthetic peptides from these two regions could affect cytolysis by HLA-A2-specific CTL (Fig. 1*b*). When a CTL line with specificity for HLA-A2 was preincubated with either of two peptides derived from residues 94-113 of the α_2 domain a significant and titratable inhibition of cytotoxicity was observed (Fig. 2*a*). The specificity of this inhibition was demonstrated in three ways. First, the peptide derived from residues 56-69 of the α_1 domain showed no inhibition, indicating that the inhibitory effect was specific to the sequence of the peptide. Second, we compared the inhibitory capacity of a peptide derived from residues 98-113 of the sequence of HLA-Aw68 with that of its HLA-A2 homologue. Within this region the two sequences only differ at position 107, where A2 has tryptophan and Aw68 has glycine[23]. The Aw68.98-113 peptide does not inhibit A2-specific CTL (Fig. 2*a*), providing further evidence for the specificity of the inhibition by peptide A2.98-113 and showing that tryptophan 107 is necessary for this activity. Third, we considered the possibility that this tryptophan might endow the peptide in a non-specific way with properties that would enable it to inhibit all CTL. To test this we measured the effects of the peptides on various clones and lines of CTL with different target specificity. The results (Fig. 2*a*,*b*; summarized in Table 1) clearly show that only CTL which are specific for HLA-A2 are inhibited by peptide A2.98-113. CTL specific for other HLA class I or class II molecules were not inhibited.

Particularly informative results were obtained with CTL clones previously characterized in analysis of HLA-Aw69[24], a recombinant molecule having an α_1 domain identical to HLA-Aw68 and an α_2 domain identical to HLA-A2[23]. Clones specific for HLA-A2 (A20.1, AI.10) and directed primarily to α_2 are inhibited by peptide A2.98-113 whereas clone AL8.1 specific for both HLA-Aw68 and HLA-Aw69 and directed primarily against α_1 is not inhibited (Table 1). Neither was clone AI5.1, which is specific for HLA-Aw69 and dependent upon a combination of the α_1 and α_2 domains, inhibited. This analysis strongly argues for the inhibition being related to the specificity of the CTL and by inference to the specificity of their T-cell receptors.

We next examined the effects of proteolytic digestion on the inhibitory capacity of the peptides. Digestion with proteinase K (Fig. 2*c*) and chymotrypsin reduced the inhibitory activity. In contrast no effect was seen with trypsin or elastase although HPLC analysis indicated that significant cleavage had occurred. Thus smaller peptides may be as potent inhibitors as A2.98-113. It may therefore be relevant that the sequences between residues 105 and 108 of all class I molecules are related to that of the fibronectin binding tetrapeptide[25]. This peptide RGDS and its reverse SDGR both have cell attachment properties[26,27]. HLA-Aw68 contain a perfect SDGR whereas in HLA-A2 the tryptophan breaks this sequence.

Our results show that residues 98-113 form an epitope recognized by the receptors of a significant proportion of the CTL stimulated by HLA-A2. This is a different part of the α_2 domain than the region from 147-157 frequently implicated from the sequences of class I mutants and variants[2-7,15-20]. These results are not incompatible as different regions may be involved, depending on CTL and the specific class I target molecule. These regions may also be adjacent in the three-dimensional structure, with both contributing to structures recognized by a single CTL. That a substitution at either position 106 or at 161 eliminates a single monoclonal-antibody-defined epitope of HLA-A2 supports this hypothesis[28,29]. The involvement of two regions, residues 98-113 and 140-160, in interaction with CTL could explain why clone AI9.1, which is specific for A2, Aw68 and Aw69 is inhibited by the HLA-A2 peptide A2.98-113 and not the HLA-Aw68 peptide Aw68.98-113 (see Table 1).

The tryptophan at position 107 that is important in the epitope seen by CTL is only found in subtypes of HLA-A2 and HLA-Aw69; all other HLA-A,B,C molecules so far analysed have glycine at this position[22]. Thus this position is one in which there is only a limited variation and in fact the region covered by peptide 98-113 contains no position at which more than two different amino acids have been found. Interestingly it is flanked by two pairs of residues (96, 98 and 114, 116) at which considerable variation occurs[22] (Fig. 1*a*).

HLA-A2 was one of the first HLA specificities to be defined and continues to be a major focus of research[30]. Three variant subtypes of HLA-A2 are distinguished by CTL and differ from the predominant form by 1-4 amino acid substitutions[15,16,20]. In addition a second group of HLA-Aw68 related molecules can be included in the HLA-A2 family[23]. Sequence comparisons within this family have enabled us to identify residues responsible for epitopes defined by monoclonal antibodies[31]. Residues 62-65 are involved in the MA2.1 epitope shared by HLA-A2 and HLA-B17 and tryptophan 107 is responsible for the PA2.1 epitope shared by HLA-A2 and HLA-Aw69[20,21,32]. But it has not been possible to demonstrate binding or inhibition of the monoclonal antibodies with peptides derived from these regions, even at concentrations far in excess of those that inhibit CTL. This is consistent with reports that the HLA-A,B,C epitopes recognized by alloantiserum, monoclonal antibodies and polyclonal xenoantisera are dependent upon conformation[33-35]. Here we show that, in contrast to the experience with antibodies, an interaction between short peptides and HLA-A2-specific CTL can be demonstrated. This distinction raises the possibility that antigen receptors of alloreactive T cells preferentially recognize degraded fragments of class I molecules. This would explain why the determinants recognized by antibodies and CTL often seem quite different[11]. A role for intact molecules would still be required to explain the potent blocking of CTL by monoclonal antibodies against conformationally dependent determinants[36,37]. It is, however, possible that both degraded and intact class I molecules are involved. This idea is attractive as alloreactive CTL recognition then becomes a special case of MHC restriction in which degraded class I molecules assume the role of nominal antigen and intact class I molecules are the restriction elements.

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Pleiotropic loss of activation pathways in a T-cell receptor α-chain deletion variant of a cytolytic T-cell clone

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Untransformed T-cell clones maintained in culture are dependent on signals transmitted through their antigen receptors (Ti; α and β chains associated with the CD3 molecules) for growth and effector function[1,2]. For cytolytic T cells (CTL), Ti stimulation also activates the killing machinery[3] and induces synthesis of gamma interferon (IFN-γ) messenger RNA and IFN-γ secretion[4]. The Thy-1 molecule, expressed on all murine cells of the T-cell lineage, has been suggested to function in transmembrane signalling, based on the ability of some anti-Thy-1 monoclonal antibodies (mAb) to activate T cells[5-9]. Recently, it was suggested that Thy-1 could function as a signal-transduction molecule when expressed in B-cell lymphomas after transfection of the gene, leading to speculation that the molecule was part of an activation pathway independent of the Ti/CD3 structures[10]. Here we report the immunoselection of a variant CTL clone which has lost expression of mRNA for the α-chain of the Ti. The Ti$^-$ variant was defective in lectin-mediated activation whether measured by increase in intracytoplasmic Ca^{2+}, CTL effector function or IFN-γ synthesis. The variant, which expressed normal levels of Thy-1, was also unresponsive to Thy-1 mAb activation as measured by IFN-γ secretion, whereas it responded to calcium ionophore plus phorbol ester. These results indicate that in a non-transformed, functional mature T-cell, Thy-1 mediated signalling is not an alternative to, but might depend on elements associated with the Ti/CD3-mediated T-cell activation pathway.

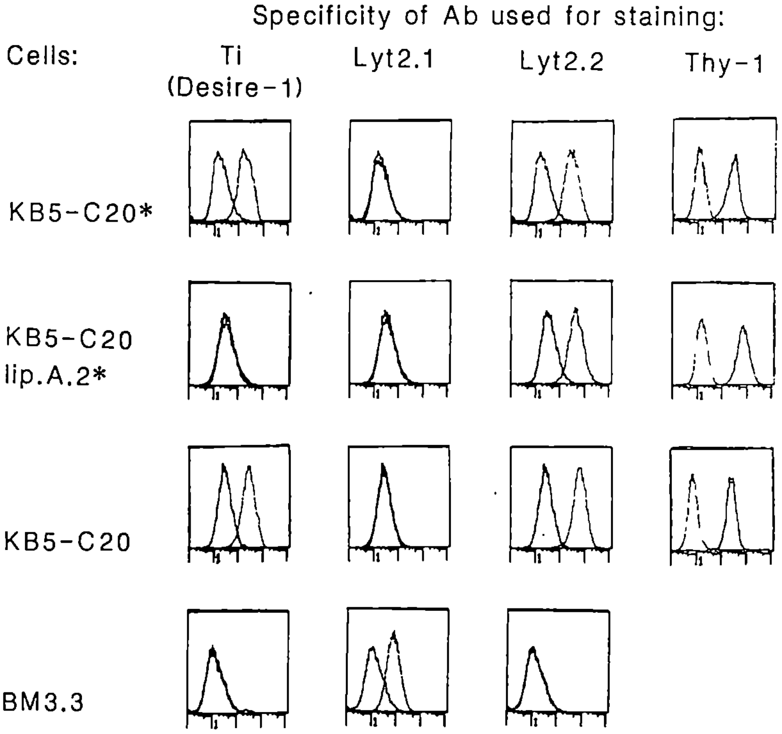

Fig. 1 Negative selection using MTX-containing protein A-coupled liposomes (MTX-lip-PA) was adapted from Machy and Leserman[13]. To 10^6 KB5-C20 cells cultured for 24 h in 1 ml 10% interleukin-2(IL-2)-containing supernatant from PMA-stimulated EL4.C16 cells[11] (SC16) were added anti-Ti mAb Désiré-1 (ref 12) (1 μg ml^{-1}) and MTX-lip-PA (final MTX concentration of 60 nM). After 24 h, the medium was replaced with 10% SC16 and a second treatment with MTX-lip-PA was performed 3 days later. Most cells had died after the two treatments and visible growth resumed after 2 weeks. A 2-h activation step using 1 μM ionomycin (Calbiochem) and 1.6×10^{-9} M PMA (Sigma) (for conditions see ref. 11) followed by replacement of the medium with 10% SC16 was performed at that time. One week later, cells were collected and transferred at 10^5 cells ml^{-1} in the presence of 10^6 2,500-rad irradiated CBA/J splenic feeder cells and 10% SC16. For immunofluorescence, T cells (2×10^5), collected 3 days after restimulation with either ionomycin/PMA and 10% SC16 (KB5-C20*, KB5-C20.lip.A.2*) or with irradiated C57BL/6 spleen cells and 10% SC16 (KB5-C20, BM3.3), were incubated in round-bottom microtitre plates for 30 min at 4 °C with 40 μl mAb at 10 μg ml^{-1}. Clone BM3.3 was used as a control, being a H-2K^b-specific CTL clone of CBA/J origin (Lyt-2.1) which does not express the Désiré-1 clonotype[12]. After 2 washes, cells were incubated with 20 μl carboxyfluorescein-containing PA-liposomes for 1 h at 4 °C, washed and fluorescence was recorded with a FACS for 10^4 cells. MAbs were Désiré-1 (anti-Ti of KB5-C20)[12], anti-Lyt-2.1 (NE1-004) and anti-Lyt-2.2 (19.178), which all bound protein A. Control samples were incubated with the protein A-binding mAb 20.8.4 (which does not bind to the H-2^k CTL clones (left curves)). Staining for expression of Thy-1 was done in one step with fluorescein-labelled anti-Thy-1.2 mAb 30.H12 (ref. 15) for 30 min at 4 °C, using a fluorescein-labelled anti-mouse IgM as a control.

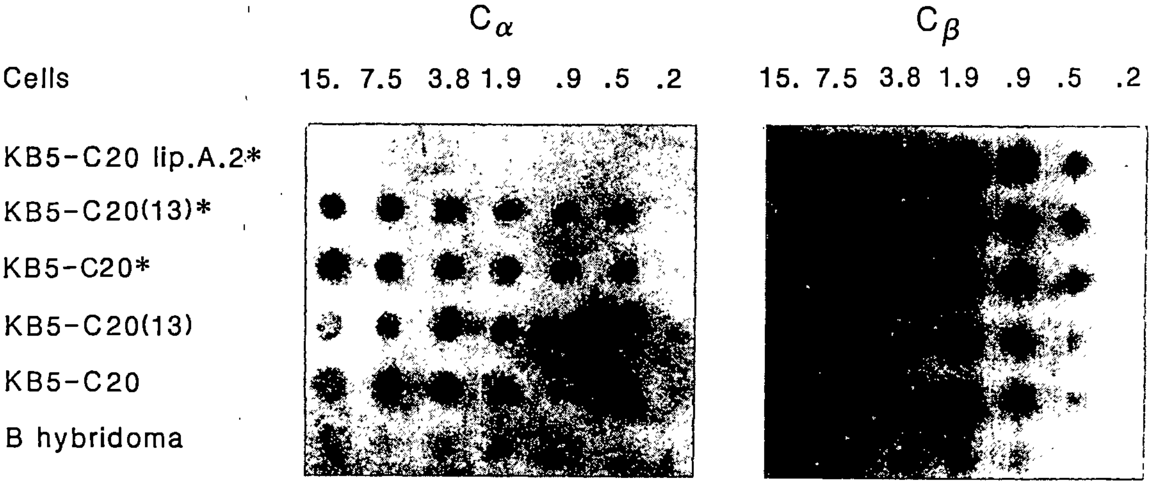

Fig. 2 RNA dot blots were prepared as described[24]. Three days after their last restimulation with ionomycin/PMA and IL-2-containing SC16 (KB5-C20 lip.A.2*, KB5-C20(13)* (a subclone of KB5-C20), clone KB5-C20*), or with irradiated C57BL/6 spleen cells and IL-2-containing SC16 (subclone KB5-C20(13) and clone KB5-C20), T-cell clones were collected on ice and washed in cold PBS. Cells from a B-cell hybridoma (100.5.28) grown in culture were used as a control. Serial dilutions of lysates, corresponding to the indicated number of cells, containing formaldehyde-denatured cytoplasmic RNA were applied to nitrocellulose filters as described[24] C_α and C_β cDNA probes corresponded respectively to 493 and 434 base pairs of the C fragments of the genes coding for the α and β chain of the T-cell receptor. The cDNAs were labelled with ^{32}P-dCTP by the hexamer priming technique, as described[25]. After prehybridization, nitrocellulose filters were hybridized with about 10^9 c.p.m. of ^{32}P-labelled cDNA probe (100 ng DNA) as described[24] and exposed to XAR5 film for 48 h. For successive hybridizations of the same filter, the ^{32}P-probe was washed off the filters by 30 min boiling in water.

We used the Ca^{2+} ionophore ionomycin and the phorbol ester phorbol myristic acetate (PMA) as substitutes for Ti-mediated activation[11], combined with immunoselection with the anti-clonotypic (anti-Ti) mAb Désiré-1[12] to isolate a CTL variant (called KB5-C20.lip.A.2) lacking surface expression of the Ti from the H-2K^b-specific clone KB5-C20 (Fig. 1). The variant was selected with methotrexate(MTX)-containing, protein A-coupled liposomes targeted to the T-cell receptor via the anti-Ti mAb using a previously described technique[13]. Northern dot blot analysis indicated that the variant lacked mRNA for the α-chain of the Ti (Fig. 2). Other cell surface markers such as Lyt-2.2, and Thy-1 (Fig. 1) or H-2K^k (results not shown) were expressed at similar levels on clone KB5-C20 and on variant KB5-C20.lip.A.2.

The availability of variant KB5-C20.lip.A.2 allowed the analysis of the influence of lack of Ti expression (and of the associated CD3 molecules (not shown)) on CTL effector functions. Results in Table 1 indicate that KB5-C20.lip.A.2 no longer lysed the H-2K^b-expressing target cell EL4.BU, which was efficiently lysed by the KB5-C20 cells (Table 1*a*). The variant also lost the

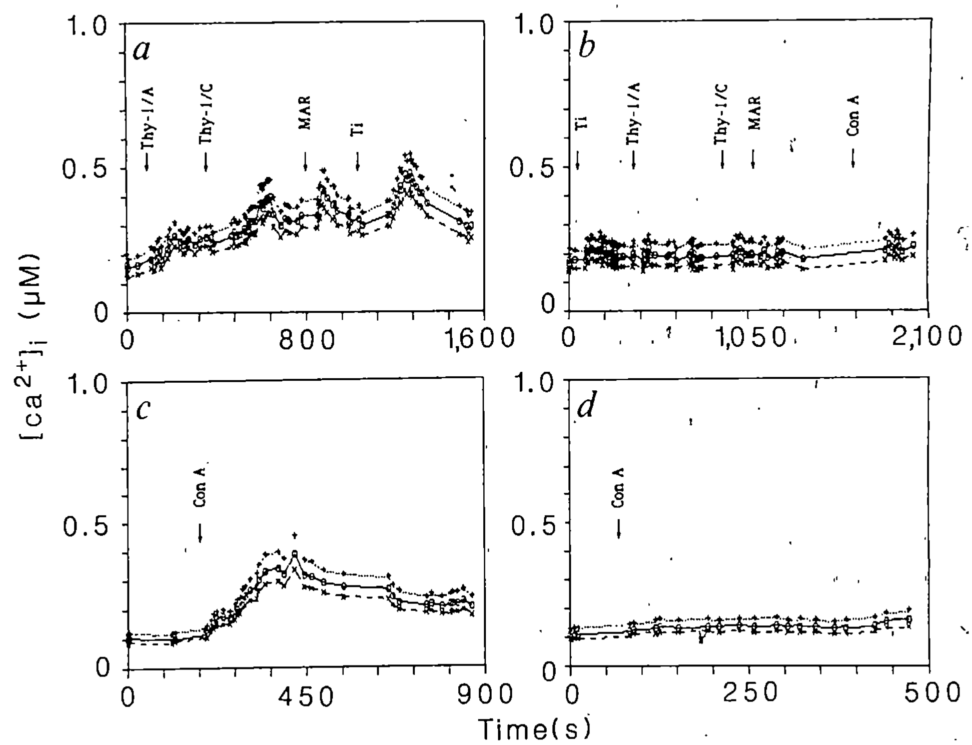

Fig. 3 Measure of intracytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$). KB5-C20 (*a*, *c*) and KB5-C20.lip.A.2* (*b*, *d*) cells were loaded with the acetoxymethyl ester form of the Ca^{2+}-sensitive dye Fura-2[26] (1 ml 1 μM Fura-2 (Molecular Probes) for 10^6 cells in RPMI+5% FCS for 30 min at 37 °C), washed and resuspended in 0.3 ml Hank's balanced salt solution (HBSS) containing 1 mM Ca^{2+} and 3% FCS. Cells were left to settle on a poly-L-lysine-coated glass cover slip placed in a 37 °C heated chamber on a fluorescence microscope as described[3]. Fluorescence was recorded on single cells at 350 and 385 nm (as described in ref. 3) at each indicated time point before and after sequential addition of mAb as indicated: Ti, 30 μg ml^{-1} mAb Désiré-1; Thy-1/A, 30 μg ml^{-1} mAb H129.93.9.1; Thy-1/C, 30 μg ml^{-1} anti-Thy-1 mAb H155.124.3; MAR, 30 μg ml^{-1} mouse anti-rat K mAb; Con A, 5 μg ml^{-1} ConA. The increase in $[Ca^{2+}]_i$ was measured for individual cells by the increase in the ratio of 350-nm to 385-nm excitation efficiency of Fura-2, which was quantified by a video silicon-intensified camera and digital image processing as described[3,27]. Mean $[Ca^{2+}]_i$ (○) was plotted as a function of time for about 50 cells. The mean was calculated with cells weighted according to their average intensity. The upper (+) and lower (×) error estimates were the weighted means of all the cells' individual upper and lower bounds in $[Ca^{2+}]_i$. Addition of ionomycin (5 μM) produced $[Ca^{2+}]_i > 1.5$ μM in all samples (not shown).

capacity to mediate antigen-independent, lectin-dependent cell-mediated cytotoxicity (LDCC) as indicated by lack of lytic activity on RDM4 target cells (Table 1*b*) in the presence of Concanavalin A (Con A), as compared to the ConA-dependent lysis exerted by KB5-C20. Similar results were obtained when phytoheamagglutinin (PHA) was used to measure LDCC (results not shown). Although participation of the Ti in LDCC had previously been suggested on the basis of blocking of LDCC by one anti-Ti mAb[14], the present results clearly establish the requirement for Ti/CD3 expression for LDCC.

To analyse whether the requirement for Ti expression was specific for the activation for killing, induction of IFN-γ secretion was used as another means of measuring activation. Results in Table 1*c* indicate that, as previously described[11], clone KB5-C20 was induced to secrete IFN-γ, as measured by macrophage-activating factor (MAF) activity, in response to either C57BL/6 cells, Con A or a combination of ionomycin and PMA. KB5-C20.lip.A.2 retained the capacity to make IFN-γ in response to ionomycin/PMA, but lost responsiveness not only to C57BL/6, but also to Con A. The basis for the enhanced efficiency of stimulation of clone KB5-C20.lip.A.2 with ionomycin alone, as compared to the requirement of both ionomycin and PMA for the stimulation of the Ti^+ clones is as yet unclear.

Binding of mAb to certain epitopes of the Thy-1 molecule has recently been shown to induce the activation programme of T-cell hybridomas[6,7] and CTL[8]. In particular, Pont *et al.*[7] described rat mAb directed against three distinct epitopes of the Thy-1 molecule such that anti-Thy-1/C and anti-Thy-1/A mAb were stimulatory in the absence and presence, respectively, of cross-linking with mouse anti-rat κ mAb (MAR), whereas anti-Thy-1/B mAb were not stimulatory. We extended these observations to CTL clones[9]. Table 1*c* shows that mAb H155.124.3 (Thy-1/C) could induce IFN-γ secretion from clone KB5-C20 but not from variant KB5-C20.lip.A.2. The monoclonal antibody H140.61 (Thy-1/B) was not stimulatory for either clone. Lack of responsiveness to anti-Thy-1 mAb cannot be attributed to absence of surface Thy-1, as detected by mAb 30.H12 (ref 15) (Fig. 1) or by mAb H155.124.3 (results not shown).

Because Ti-[16], Con A-[17] and Thy-1[10]-mediated activation have in common an initial increase in internal calcium concentration $[Ca^{2+}]_i$ we analysed this parameter on the Ti-negative variant. Results in Fig. 3*a* indicate that clone KB5-C20 responded with an increase in $[Ca^{2+}]_i$ after addition of anti-Thy-1 mAb H129.93.9.1 (Thy-1/A) and H155.124.3 (Thy-1/C) followed by MAR. These mAb have been shown to act synergistically in T-cell activation[7]. Similar results were obtained with clone KB5-C20* (maintained in culture by stimulation with ionomycin and PMA in the same manner as clone KB5-C20.lip.A.2*) (results not shown). Both Ti^+ cells also increased their $[Ca^{2+}]_i$ in response to anti-Ti mAb Désiré-1 (Fig. 3*a*) and the response was further increased after addition of a rabbit anti-mouse immunoglobulin reagent (result not shown). No change in $[Ca^{2+}]_i$ was observed on binding of rat anti-Lyt-2 mAb (53.6.7) followed by MAR to either KB5-C20, KB5-C20* or KB5-C20.lip.A.2* (results not shown). ConA alone induced an increase in $[Ca^{2+}]_i$ in the two Ti^+ KB5-C20 clones (Fig. 3*c* and results not shown). The Ti^- clone KB5-C20-lip.A.2, however, was totally unresponsive, as measured by $[Ca^{2+}]_i$ to the Désiré-1 anti-Ti mAb, the combination of anti-Thy-1 mAb followed by MAR (Fig. 3*b*), and also to ConA (Fig. 3*b*,*d*).

The simultaneous loss of expression of the Ti structure and of activation induced by either Thy-1-specific mAb or ConA was also observed for sub-clones derived from KB5-C20-lip.A.2 as well as for three other variants obtained independently with the same immunoselection technique (not shown), whereas Ti^+ subclones of KB5-C20 were always found to be responsive. The recurrence of the observation strongly suggests that there is a pleiotropic loss in activation pathways rather than a concomitant, random selection for loss of Ti expression and loss of Con A and Thy-1-mediated activation pathways. Attempts at reexpressing the α-chain mRNA after transfection of the gene are

Table 1 Characteristics of the Ti^- variant KB5-C20.lip.A.2

a, H-$2K^b$-specific CTL activity measured on EL4 target cells:

E:T	KB5-C20	KB5-C20*	KB5-C20.lip.A.2*
5:1	52	25	0
1:1	25	10	0

b, Con A-induced CTL activity measured on RDM4 target cells:

E:T	KB5-C20		KB5-C20*		KB5-C20.lip.A.2*	
	−ConA	+ConA	−ConA	+ConA	−ConA	+ConA
10:1	−0.2	46	−0.4	29	2.2	6.0
3:1	0.0	28	1.6	28	2.6	2.0
1:1	−0.3	12	0.8	15	1.5	2.6
0.3:1	1.0	6.0	−0.5	10	−0.7	0.2

c, MAF units in supernatants from CTL clones:

	KB5-C20		KB5-C20.lip.A.2*	
Stimulated with	−PMA	+PMA	−PMA	+PMA
Control	<3	<3	<3	<3
Ionomycin	<3	50	55	290
CBA spleen cells	<3	<3	<3	<3
C57BL/6 spleen cells	74	77	<3	<3
Anti-Ti mAb	54	118	<3	<3
Anti-Thy-1/C mAb	300	160	<3	<3
Anti-Thy-1/B mAb	<3	<3	<3	<3
Concanavalin A	240	150	<3	<3

In *a* and *b*, cytolytic activities of clones KB5-C20, KB5-C20* and KB5-C20.lip.A.2* were measured in a 5 h ^{51}Cr-release assay as described[12] on the H-2^b EL4.BU (*a*) or on the H-2^k RDM4 (*b*) target cells untreated for direct lysis or preincubated for 30 min at 37 °C with 10 μg ml^{-1} Con A for lectin-dependent lysis (LDCC). The percentage specific ^{51}Cr release (mean of triplicate samples) from 10^4 target cells is shown for different effector to target cell ratios (E:T). In *c*, macrophage activating factor (MAF) was measured as described[11] in supernatants from 10^5 clone cells incubated for 20 h at 37 °C in 96-well flat-bottomed microtitre plates in 0.2 ml medium in the absence (−PMA) or in the presence (+PMA) of 1.6×10^{-9} M PMA. In all conditions of stimulation, MAF was a measure of interferon gamma (IFN-γ) as indicated by inhibition of MAF activity with IFN-γ specific mAb (A.G., C.B. and M.B., unpublished results). Stimulation conditions of the CTL clones were as follows: ionomycin, 2 μM; spleen cells from CBA/J(H-2^k) or from C57BL/6 (H-2^b) mice, 10^6 cells irradiated at 2,000 rad; anti-Ti mAb: 30 μg ml^{-1} Désiré-1[12]; anti-Thy-1/C mAb, 30 μg ml^{-1} H155.124.3 (ref. 7); anti-Thy-1/B mAb, 30 μg ml^{-1} H140.61.2 (ref. 7); ConA, 5 μg ml^{-1} ConA.

in progress. The finding of lack of lectin responsiveness of the Ti^- variant extends to a non-transformed CTL the observation by Weiss and colleagues on the lack of PHA stimulation of Tiβ-chain-deficient Jurkat cells[18], a property which could be recovered after transfection of the β-chain gene[19]. It furthermore indicates that among the different CTL cell surface glycoproteins which bind ConA[20], only the Ti complex effectively transduces intracellular signals, whether measured by early $[Ca^{2+}]_i$, by CTL effector function, or by IFN-γ synthesis.

The striking result reported here is the apparent dependence of the Thy-1-mediated activation pathway on expression of the Ti complex. This is surprising in view of the fact that the Thy-1 molecule was not coprecipitated with the Ti–CD3 complex from clone KB5-C20 (ref. 12) and that the two molecules could be modulated independently from the cell surface[9]. There is not necessarily a contradiction between our results and the finding[10] that the Thy-1-molecule expressed on a B lymphoma after gene transfer manifested an increase in $[Ca^{2+}]_i$ in response to activating Thy-1-specific mAb. That experiment does not prove the independence of the Thy-1-mediated signalling pathway as surface immunoglobulin or associated structures on B cells might replace the function of the Ti–CD3 complex on T cells. Using human T cells, O'Flynn and colleagues[21] suggested that PHA activation was mediated by the CD2-dependent pathway. The latter conclusion, being based only on mAb blocking data using cells also expressing the Ti–CD3 complex, is probably not definitive. However, a subpopulation of human thymocytes expressing the CD2 antigen in the absence of Ti/CD3 surface expression appears to be stimulated through the CD2 pathway[22]. No equivalent to the human CD2 molecule is known in the mouse, but a similar distribution on immature and mature T cells suggested that in the mouse the Thy-1 molecule might be part of a signalling pathway distinct from the Ti-mediated pathway and possibly used for activation of cells of the T lineage before surface expression of the Ti complex[10]. Results presented here clearly show that on mature functional T cells, the Thy-1 structure does not function as an alternative activation pathway to the Ti complex. It remains to be determined whether the dependence of Thy-1-mediated activation on the Ti/CD3 complex is at the level of the generation of inositol trisphosphate leading to Ca^{2+} mobilization[23] or at the level of Ca^{2+} influx through a channel possibly associated with the Ti complex.

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A novel c-*abl* protein product in Philadelphia-positive acute lymphoblastic leukaemia

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Activation of cellular proto-oncogenes as a result of chromosomal abnormalities has been implicated in the development of some human malignancies. Perhaps one of the most striking examples of this association occurs in chronic myelogenous leukaemia, where the Philadelphia (Ph) translocation results in substitution of the 5′ end of the c-*abl* proto-oncogene with *bcr* gene sequences. A unique hybrid *bcr-abl* message is produced. As the Ph translocation is also present in some patients with acute lymphoblastic leukaemia, we initiated studies to determine if similar genomic events occur in these two different forms of Ph-positive leukaemia. Here we report that the Ph translocation in acute lymphoblastic

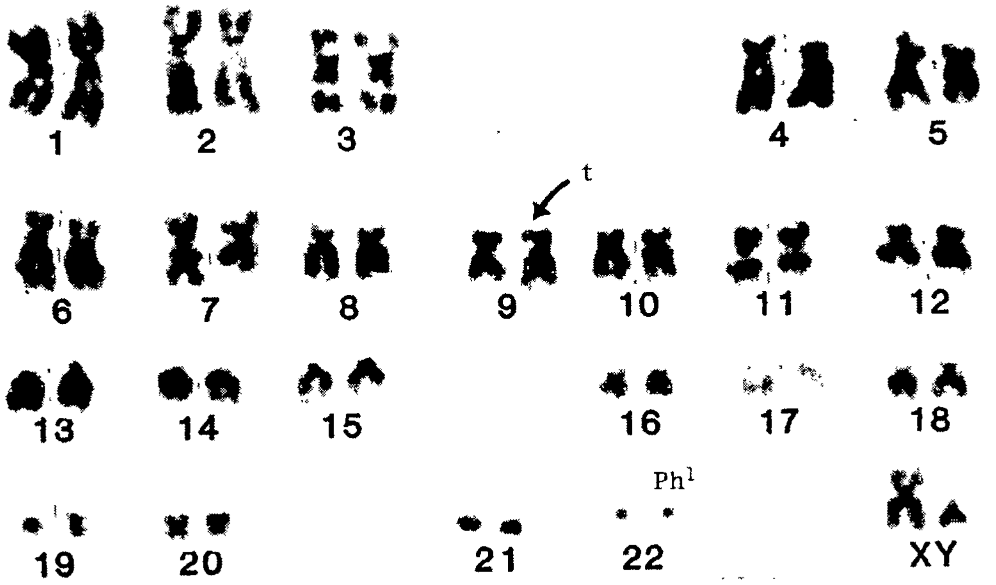

Fig. 1 Karyotype of Ph-positive acute lymphoblastic leukaemia patient 2. Giemsa banded chromosomes of the bone marrow cells show a pseudodiploid clone 46, XY, t(9;22)(q34;q11).

Methods. Standard cytogenetic procedures were used; slide preparations were stained with Giemsa stain after trypsin pretreatment, yielding G-banded chromosomes[29].

leukaemia can result in production of a novel aberrant c-abl protein that is distinct from the bcr-abl protein found in Ph-positive chronic myelogenous leukaemia. Our observations suggest that alternative mechanisms of activation of c-*abl* exist, and may be important in the development of human acute lymphoid rather than chronic myeloid malignancies.

In 1960, Nowell and Hungerford discovered a shortened chromosome 22, designated the Philadelphia (Ph) chromosome, in chronic myelogenous leukaemia (CML)[1]. This chromosomal aberration, which is now considered the cytogenetic hallmark of the disease, results from a reciprocal translocation between chromosomes 9 and 22, [t(9; 22)(q34; q11)][2,3]. The breakpoints on chromosome 22 occur within a 5.8 kilobase (kb) segment of DNA, termed the breakpoint cluster region or *bcr*[4,5]. A crucial event in the Ph translocation is the transfer of the c-*abl* proto-oncogene from its normal residence on chromosome 9 to chromosome 22, so that *bcr* and c-*abl* sequences are juxtaposed in a head-to-tail configuration with *bcr* closer to the centromere[6-8]. The resulting mRNA is a hybrid of 5′ *bcr* sequences and a truncated c-*abl* gene lacking the first exon[8,9]. This chimaeric 8.5-kb *bcr-abl* transcript encodes an aberrant protein of relative molecular mass 210,000 (M_r 210K), whereas the normal c-*abl* gene is expressed as 6- and 7-kb messenger RNAs encoding a 145K protein[10-13]. Both p210$^{bcr\text{-}abl}$ and its normal homologue, p145$^{c\text{-}abl}$, as well as the Abelson murine leukaemia virus-encoded p160$^{gag\text{-}abl}$ have an associated tyrosine protein kinase activity. However, p160$^{gag\text{-}abl}$ and p210$^{bcr\text{-}abl}$ probably use themselves as substrates in a different way from p145$^{c\text{-}abl}$ *in vitro*[12]. The high specificity and altered autophosphorylation properties of p210$^{bcr\text{-}abl}$ strongly imply that this molecule is critical in the pathogenesis of CML.

Interestingly, the Ph chromosome has also been detected in ~17–25% of adult patients with acute lymphoblastic leukaemia (ALL)[14,15]. It is not known whether the presentation as acute leukaemia or CML represents a different clinical manifestation of the same malignant process—Ph positive leukaemia—or, despite the presence of an identical cytogenic abnormality, a distinction should be drawn between the two disorders. A strong argument supporting a single underlying disease process for at least some cases of Ph-positive ALL and CML are the reports of patients presenting with ALL in whom transition to CML has been documented[16]. In addition, ~$\frac{1}{3}$ of patients with CML will eventually develop a lymphoid blast transformation[17]. This clinical state strongly resembles ALL. Nevertheless, these two disorders have fundamental differences, with the salient feature of CML being the rapid proliferation of mature myeloid cells whereas that of ALL is a greatly increased number of immature lymphoid cells. These unique clinical manifestations of Ph-positive CML compared with ALL suggest discrete events in their pathogeneseis.

As genetic events are assumed to be critical determinants of the phenotypic manifestations of disease, we have studied c-*abl* expression and searched for *bcr* rearrangement in two Ph-positive ALL patients. The diagnosis of ALL was based on several features: (1) presentation with >80% blasts in a hypercellular bone marrow; (2) lymphoblastoid-appearing cells by light microscopy; and (3) positive terminal deoxynucleotidyl trans-

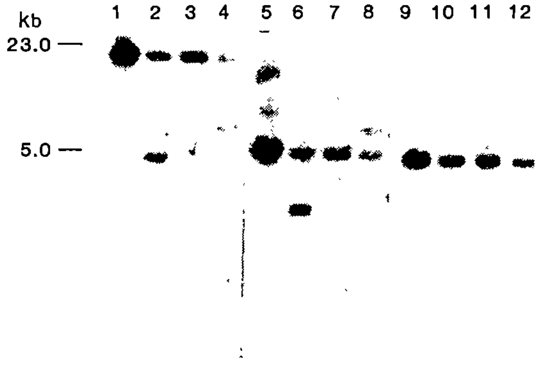

Fig. 2 Southern blot analysis of patient DNA samples and hybridization with a genomic *bcr* probe[4]. Restriction enzymes used were *Eco*RI (lanes 1–4), *Bbl*II (lanes 5–8) and *Hin*dIII (lanes 9–12). Lanes 1, 5, 9: normal granulocytes; lanes 2, 6, 10: Ph-positive ALL patient 1; lanes 3, 7, 11: Ph-positive ALL patient 2; lanes 4, 8, 12: Ph-positive CML patient.

Methods. DNA was prepared by cell lysis, proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation as described[30]. Genomic DNA (20 μg) was digested with restriction endonucleases (2 units per μg DNA) in conditions recommended by the supplier (International Biotechnologies, Inc.), size fractionated by electrophoresis in a 0.8% agarose gel, denatured and transferred to nitrocellulose filters as described by Southern[31]. A 3′, 1.2 kb *bcr* (*Hin*dIII-*Bgl*II) plasmid probe (Oncogene Science, Inc.) was used[4]. The probe was nick-translated to a specific activity of $1\text{–}2\times10^8$ c.p.m. per μg DNA. Hybridization was carried out overnight at 42 °C in the presence of 10% dextran sulphate. The filters were then washed at 60 °C for 1 h in 0.1×SSC (1×SSC = 0.15 M sodium chloride/0.015 M sodium citrate, *p*H 7) containing 0.1% sodium dodecyl sulphate (SDS), air dried and autoradiographed.

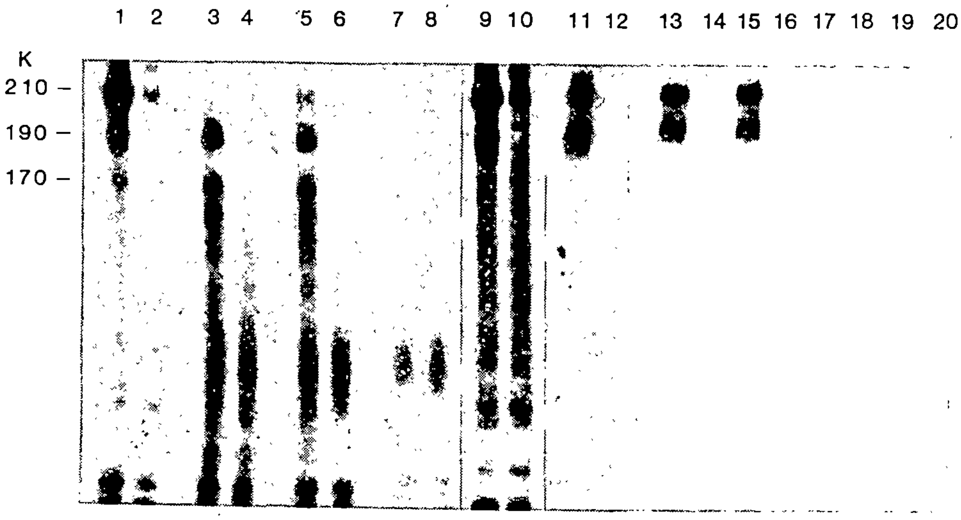

Fig. 3 Immune complex kinase assays. Antisera used were anti-abl 389-403 (lanes 1, 2, 3, 4, 13, 14, 17, 18), anti-abl3 (lanes 5, 6, 9, 10) and anti-bcr3 (lanes 7, 8, 11, 12, 15, 16, 19, 20). In even lanes, the reactivity of the antisera was blocked by addition of cognate peptide. Lanes 1, 2, 9-12: K562 cells (CML cell line); lanes 3-8: Ph-positive ALL patient 2; lanes 13-16: Ph-positive ALL patient 1; lanes 17-20: Ph-negative ALL patient.

Methods. Antisera were made against peptides representing the predicted hydrophilic domain of v-abl (anti-abl 389-403)[10], a predicted high term potential domain of c-abl based on the Chou–Fasman predictive model for protein secondary structure[32] and a region within *bcr*, exon 3[8]. Methods were as previously described[10]; briefly, peptides were made using solid phase synthesis; cysteine residues were added to the carboxyl termini during synthesis for MBS cross-linking through free sulphydryl groups to the carrier protein keyhole limpet haemocyanin and antigen was prepared by emulsifying at 1:1 ratios 0.2 mg of cross-linked peptide in Dulbecco's phosphate buffered saline with 1.5 ml of Freund's adjuvant. Subcutaneous injections of rabbits were administered at multiple sites. Three booster injections of antigen suspended in incomplete Freund's adjuvant were given at two-week intervals. Blood samples were prepared for immune complex kinase assays by treating with 5 volumes of 1.22% ammonium oxalate to lyse red blood cells and Ficoll Hypaque gradient centrifugation to enrich for immature cells. Each sample was divided into aliquots of 5×10^7 cells. Each aliquot was disrupted with 1 ml of lysis buffer (1% Triton X-100, 5 mM EDTA, 100 mM NaCl, 5 mM phenylmethylsulphonylfluoride, 100 KIU ml^{-1} Trasylol, in 10 mM sodium phosphate, *p*H 7.5) with 20 strokes in a tight-fitting Wheaton homogenizer. Lysates were subjected to a 10,000*g* centrifugation for 10 min at 4 °C. The supernatants were then incubated with 5 μl antisera for odd lanes, and a mixture of 5 μl antisera and 5 μl of 1 mg ml^{-1} cognate peptide for even lanes, for 1 h on ice. The resulting immune complexes were then precipitated by incubation with 10 μl of Pansorbin for 20 min on ice. Immunoprecipitates were washed twice with wash buffer (0.1% Triton X-100, 150 mM NaCl, in 10 mM sodium phosphate, *p*H 7.5). The pellets were drained, resuspended in 50 μl of 20 mM HEPES (*p*H 7.0), containing 0.1% Triton X-100 and 150 mM NaCl, then reacted with 50 μl of a mixture containing 5 μCi [γ-^{32}P] ATP (50 Ci $mmol^{-1}$), 20 mM $MnCl_2$, 0.1% Triton X-100 in 20 mM HEPES (*p*H 7.0), for 10 min on ice. The reaction was terminated by addition of 3 ml of buffer containing 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate (DOC), 100 mM NaCl, 5 mM EDTA, in 10 mM sodium phosphate, *p*H 7.5. The immune complexes were washed twice with the above buffer and then prepared for electrophoresis according to Laemmli[33]. Phosphoproteins were electrophoretically resolved according to molecular weight on a 8% sodium dodecyl sulphate polyacrylamide gel and sized with prestained markers. The gel was exposed overnight on Kodax XAR-5 film.

ferase and negative myeloperoxidase in immunocytochemical stains of bone marrow cells[18]. In patient 1, cytogenetic analysis revealed 45, XX, −7,t(9;22)(q34;q11) in 100% of bone marrow metaphases; in patient 2, 46,XY,t(9;22) (q34;q11) (Fig. 1).

To determine whether the 22q11 breakpoints occurred within *bcr*, we performed Southern blot analysis using a 3′ 1.2-kb *Hin*dIII-*Bgl*II genomic *bcr* probe (Oncogene Science). Rearrangement in *bcr* in DNA derived from ALL patient 1 was observed after digestion with restriction enzymes *Eco*RI, *Bgl*II, and *Bam*HI, but not *Hin*dIII (Fig. 2, lanes 2, 6 and 10, and data not shown). A similar pattern of rearrangement, with localization of the breakpoint between exons 2 and 4 of *bcr*, was seen in 7 of 7 CML patients in lymphoid blast crisis (M. Shtalrid, unpublished observations) and in 8 of 8 CML patients in benign phase or myeloid blast crisis. Representative data are shown in Fig. 2, lanes 4, 8 and 12. In contrast, rearrangement in *bcr* was not detected in Ph-positive ALL patient 2 (Fig. 2, lanes 3, 7 and 11). As the C_λ gene resides at 22q11[19], we reprobed the filters with a genomic C_λ probe[20]. Rearrangement was not observed (data not shown), indicating that the breakpoint on chromosome 22q11 in ALL patient 2 does not occur within the *bcr* or C_λ gene. These results are consistent with recent work by Erikson *et al.*[21] showing a lack of *bcr* rearrangement in 3 of 5 Ph-positive ALL patients. Using *in situ* hybridization on cells obtained from one of these patients, they determined that the break at 22q11 occurred between the C_λ and the *bcr* gene. Therefore, the location of the breakpoint on chromosome 22 in a significant proportion of Ph-positive ALL patients may be different from that in Ph-positive CML patients, despite an identical cytogenetic marker.

As the protein products of genes are the molecules ultimately responsible for phenotype, it was of interest to examine the c-abl protein expressed in these two different Ph-positive ALL patients. Therefore, immune complex kinase assays using synthetic peptide sera developed against the predicted hydrophilic domain of v-abl (anti-abl389-403)[10], a predicted high term potential region within c-abl (anti-abl3), and a region within *bcr* exon 3 (anti-bcr3), were performed. A 210K protein, $p210^{bcr\text{-}abl}$, was detected in K562 cells (CML blast crisis cell line) by the two anti-abl sera and by the anti-bcr serum (Fig. 3, lanes 1, 2 and 9-12). The 190K protein in K562 cells, detected in variable amounts by these three antisera, is felt to represent a proteolytic fragment of $p210^{bcr\text{-}abl}$ (ref. 11). The protein $p210^{bcr\text{-}abl}$ was also present in 14 of 14 (100%) Ph-positive CML patients, three of whom were in lymphoid blast crisis, and in Ph-positive ALL patient 1 (Fig. 3, lanes 13-16 and data not shown). In contrast, $p210^{bcr\text{-}abl}$ was not seen in Ph-positive ALL patient 2, who did not have *bcr* rearrangement. Unexpectedly, proteins of M_r 190K and 170K were found in this patient using both anti-abl389-403 and anti-abl3 sera (Fig. 3, lanes 3 and 5). These proteins were abl-specific as they were not detected in assays in which the reactivity of the anti-abl sera was blocked by prior addition of cognate peptide (Fig 3, lanes 4 and 6). Furthermore, detection of p190 and p170 by two anti-abl sera, made against different domains within the abl protein, provides cogent evidence that these are bonafide c-abl proteins. The p190 observed in ALL patient 2 is distinct from the 190K proteolytic fragment seen in Ph-positive CML, since anti-bcr3 serum does not detect the former protein (Fig. 3, lane 7), but clearly reacts with the latter protein (Fig. 3, lane 11). The amount of p170 relative to p190 varied in different repeats of the experiment, suggesting that p170 may be a proteolytic fragment of p190. The protein p190 was not found in a Ph-negative ALL patient (Fig. 2, lanes 17-20), supporting a unique association between this

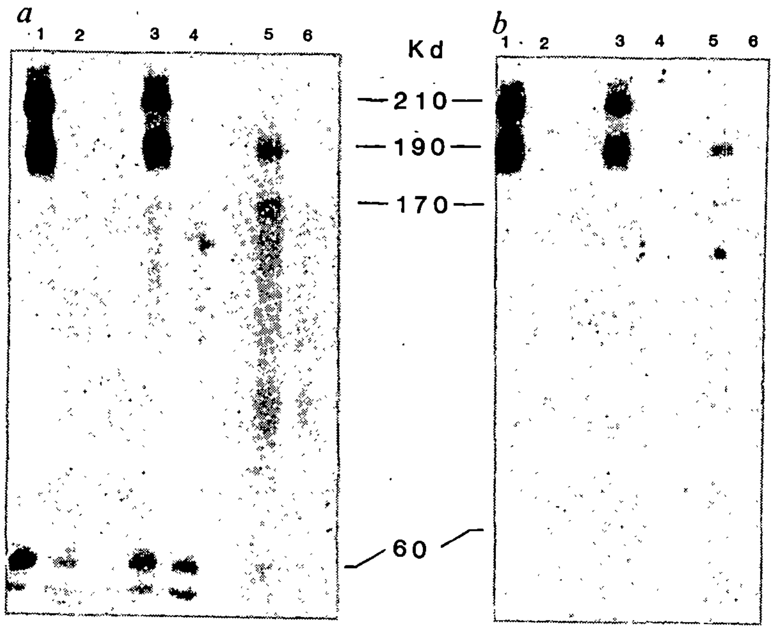

Fig. 4 Immune complex kinase assays *a*, before and *b*, after KOH hydrolysis of the gels. Antisera used were anti-bcr3 (lanes 1, 2) and anti-abl389-403 (lanes 3-6). In even lanes, the reactivity of the antisera was blocked by incubation with cognate peptide. Lanes 1-4, K562 cells; lanes 5 and 6, Ph-positive ALL patient 2.

Methods. Methodology for immune complex kinase assays was identical to that described for Fig. 3. Alkali treatment of gels was performed as follows[22]. After the dried gel was autoradiographed (*a*), it was swollen in 15 volumes of 1 M KOH and incubated at 55 °C for 2 h. The gel was then neutralized and washed with gentle shaking, with four changes of 10% acetic acid + 10% isopropanol for at least 2 h. The gel was then dried again and autoradiographed (*b*).

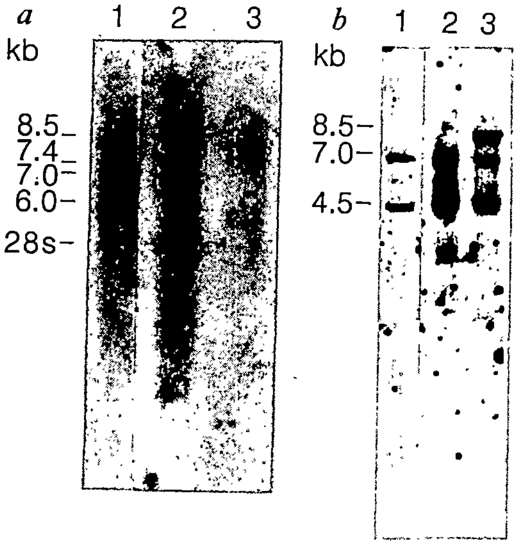

Fig. 5 Northern blot analysis of mRNA from patient samples and hybridization with a genomic c-*abl* probe[23] (*a*) and a *bcr* cDNA probe[8] (*b*). Lane 1, Ph-negative acute promyelocytic leukaemia cell line HL-60; lane 2, Ph-positive ALL patient 2; lane 3, Ph-positive CML patient.

Methods. RNA was prepared using the guanidinium/caesium chloride method[34]. Polyadenylated RNA was selected by chromatography on oligo (dT)-cellulose columns. The polyadenylated RNA (10 μg per lane) was size fractionated by electrophoresis in 1.1% agarose gels containing 2.2 mol l^{-1} formaldehyde, transferred to nitrocellulose filters with 20 × SSC, and hybridized to radiolabelled ^{32}P-DNA probes at 42 °C for 12 h by a modification of a described method[35]. The probes were radiolabelled by nick translation to a specific activity of $1\text{-}3 \times 10^8$ c.p.m. μg^{-1} of DNA. The filters were then washed in 0.1 × SSC, dried, and autoradiographed with intensifying screens for seven days at −70 °C. The c-*abl* probe was derived from a subclone of a c-*abl*-containing cosmid. We used an *Eco*RI-*Bam*HI fragment corresponding to the most 5′ v-*abl* hybridizing region[23]. To probe for *bcr* we used a *bcr* cDNA probe (0.45-kb *Eco*RI-*Pst*I fragment)[8]. An aberrant 7.4-kb c-*abl* transcript was detected in the Ph-positive ALL cells (*a*, lane 2). In contrast, the characteristic 8.5-kb *bcr-abl* mRNA was detected in the Ph-positive CML cells by both the c-*abl* and *bcr* probe (*a* and *b*, lane 3). Only the normal 4.5- and 6.7-kb *bcr* was present in the ALL cells (*b*, lane 2) confirming the absence of these *bcr* sequences in the aberrant 7.4-kb c-*abl* message. All lanes were run on the same gel.

protein and Ph-positive ALL; p190 and p170 remained visible after KOH hydrolysis[22] of the gel consistent with phosphorylation occurring on tyrosine (Fig. 4).

Further evidence confirming the novel nature of c-*abl* expression in ALL patient 2 was obtained from Northern blot analysis (Fig. 5*a*). Hybridization with a genomic c-*abl* probe[23] revealed the characteristic 8.5-kb *bcr-abl* mRNA in a Ph-positive CML patient whereas Ph-positive ALL patient 2 expressed an aberrant 7.4-kb c-*abl* transcript. The 7.4-kb mRNA was not detected after rehybridization of the same filters with a *bcr* complementary DNA (0.45-kb *Eco*RI-*Pst*I fragment) probe[8] (Fig. 5*b*), confirming the absence of these *bcr* sequences in this aberrant c-*abl* message. The difference in size between the aberrant transcripts found in the ALL and CML patients is consistent with production of a 190K rather than a 210K protein.

Our observations suggest that p190 is a novel aberrant c-*abl* protein product. As a similar pattern of c-*abl* expression was not observed in multiple CML patients, including those whose disease evolved to lymphoid blast crisis, p190$^{c\text{-}abl}$ may be uniquely associated with development of acute lymphoid rather than chronic myeloid leukaemia. In this regard, it is of interest that v-*abl* causes transformation of lymphoid cells *in vitro*[24] and murine lymphomagnesis *in vivo*[25]. The common *abl* exon is an acceptor for splices from at least four 5′ c-*abl* exons[26], as well as two *bcr* exons[27]. The substitution of the normal 5′ end of *abl* with *bcr* sequences is presumed to convert c-*abl* from a proto-oncogene to an oncogene, just as *gag* sequences do for v-*abl*[28]. Therefore, the pivotal differences between v-*abl* and *bcr-abl* as opposed to c-*abl* lie in the replacement at the amino terminus. It is possible, that the amino termini of p190$^{c\text{-}abl}$ and p160$^{gag\text{-}abl}$ have undergone substitution with sequences that activate the tumorigenic potential of these molecules for lymphoid rather than myeloid cells. Alternatively, the rearrangement leading to p190$^{c\text{-}abl}$ may be one which is favoured in lymphoid cells. In this case, the nature of the N-terminal sequences appended to the residual c-*abl* sequences may be irrelevant to its activation, with the critical event being the loss of regulatory N-terminal c-*abl* sequences.

Our study demonstrates that similar chromosomal abnormalities do not necessarily yield identical genomic events. At least two mechanisms of activation of c-*abl* exist as a result of the Ph translocation in ALL: (1) c-*abl* may be fused with the *bcr* moiety in a configuration indistinguishable from that found in Ph-positive CML; (2) c-*abl* may be fused with other, as yet undetermined sequences. These sequences could include an upstream *bcr* fragment, or another gene, instead of *bcr*. It may be that Ph-positive ALL represents a nosologic continuum, with those patients in whom the salient molecular characteristic is formation of a bcr-abl protein representing the end-stage of CML which escaped detection during its early, often asymptomatic, benign phase; other patients, in whom alternate mechanisms of activation of c-*abl* occur, may be predisposed to presentation with acute lymphoid leukaemia without a preceding benign myeloid phase. Further investigation of c-*abl* expression in Ph-positive ALL is in progress and should help elucidate the role of molecular variants of this gene in acute lymphoid and chronic myeloid tumorigenesis.

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A novel *abl* protein expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia

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The Philadelphia (Ph) chromosome breakpoints in chronic myelocytic leukaemia are clustered on chromosome 22 band q11 in a 5.8-kilobase (kb) region designated *bcr*[1]. The c-*abl* proto-oncogene is translocated from chromosome 9 band q34 into *bcr* and the biochemical consequence of this molecular rearrangement is the production of an abnormal fusion protein *bcr–abl* p210 with enhanced protein-tyrosine kinase activity[2,3] compared to the normal p145 c-*abl* protein. The Ph chromosome translocation is also seen in some acute lymphoblastic leukaemias with B-cell precursor phenotypes[4,5] some of which have *bcr* rearrangement (bcr$^+$) and some do not (bcr$^-$)[6,7]. We present evidence that the Ph$^+$, bcr$^-$ leukaemias are associated with a novel p190 *abl* kinase. We propose that acute lymphoblastic leukaemias that are bcr$^+$, p210$^+$ are probably lymphoid blast crises following a clinically silent chronic phase of chronic myelocytic leukaemia arising in multipotential stem cells whereas bcr$^-$, p190$^+$ cases are *de novo* acute lymphoblastic leukaemias arising in more restricted precursors.

We obtained four cases of Ph$^+$ acute lymphoblastic leukaemia leukaemia (ALL) for our study (F.B., F.Y., P.N. and A.E.). The Ph chromosome was observed by cytogenetic analysis in each case. Immunophenotyping of the blast cells revealed a common ALL phenotype[8] in three patients (F.B., F.Y., P.N.) and an unclassifiable phenotype in patient A.E. (co-expression of lymphoid and myeloid antigens).

Table 1 Summary data of Ph$^+$ acute lymphoblastic leukaemia cases

Patient	Age/sex	Ph chromosome	*bcr*	*abl* protein
F.B.	54/F	+	Rearranged	p210
F.Y.	35/F	+	Normal	p190
P.N.	46/M	+	Normal	p190
A.E.	14/M	+ (9; 17; 22)*	Normal	p190

* Banding was performed on case A.E., which showed the same chromosomal breakpoints on chromosome 9 and 22 (bands 9q34 and 22q11) as in Ph$^+$ CML, although the cells were unusual in having a complex variant translocation involving chromosomes 9, 17 and 22.

The *bcr* status in the leukaemic DNA was determined using a large *bcr* probe (Fig. 1*a*) and four different digests, *Bgl*II, *Bam*HI, *Hin*dIII and *Eco*RI. Rearrangement of *bcr* was observed in the DNA from patient F.B. while only the normal restriction enzyme fragments were present in DNA from patients F.Y., P.N. and A.E. (Fig. 1*b*, and data not shown). These results confirm earlier reports that Ph$^+$ ALL is heterogenous with respect to involvement of the *bcr*[6,7], in contrast to Ph$^+$ chronic myelocytic leukaemia (CML), where *bcr* rearrangement has been observed in all 40 cases we have examined (data not shown).

As the 5.8-kb *bcr* is not involved in 3 out of the 4 Ph$^+$ ALL, it was important to investigate whether *abl* was affected by the translocation event. Using an *abl*-specific affinity purified polyclonal antiserum, *abl*-related proteins were immunoprecipitated from leukaemic cells of the four Ph$^+$ ALL cases. The *in vitro* phosphorylated immunoprecipitates from cells of patient F.B. (Ph$^+$ bcr$^+$) or Nalm-1 (a cell line derived from a CML lymphoid blast crisis[9] which is also Ph$^+$, bcr$^+$) revealed a phosphoprotein of relative molecular mass 210,000 (M_r 210K) called p210, which is the appropriate size for the *bcr–abl* fusion protein (Fig. 2*a*). In contrast, a phosphoprotein of M_r 190 K (p190) was seen in each of the three Ph$^+$, bcr$^-$ ALL patients (Fig. 2*a*). In control experiments, neither p210 nor p190 was observed in two Ph$^-$,

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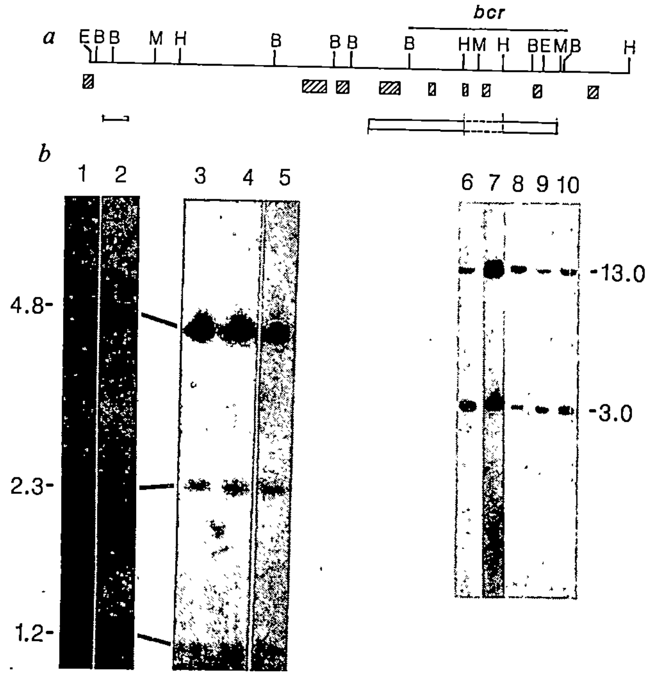

Fig. 1 DNA analysis to detect *bcr* rearrangements. *a*, Restriction map of the characterised *bcr* of chromosome 22 band q11. Hatched boxes, known positions of exons; open box, *bcr* probe (the dotted region was deleted because it contains repetitive sequences). The line above the map denotes the region designated *bcr*. Restriction enzyme sites shown thus: E, *Eco*RI; B, *Bgl*II; M, *Bam*HI; H, *Hin*dIII. Scale bar, 1 kb. *b*, DNA was digested with *Bgl*II (lanes 1-5) or *Bam*HI (lanes 6-10), electrophoresed through a 0.7% agarose gel, transferred to nitrocellulose[16] and allowed to hybridize to the *bcr* probe depicted in *a*. DNAs were isolated from (lanes 1 and 6) control cells of Bri-7, an EBV transformed cell line without the Ph chromosome; lanes 2 and 7, cells from patient F.B.; lanes 3 and 8, cells from patient F.Y.; lanes 4 and 9, cells from patient P.N.; and lanes 5 and 10, cells from patient A.E. Sizes of unrearranged bands in kilobases.

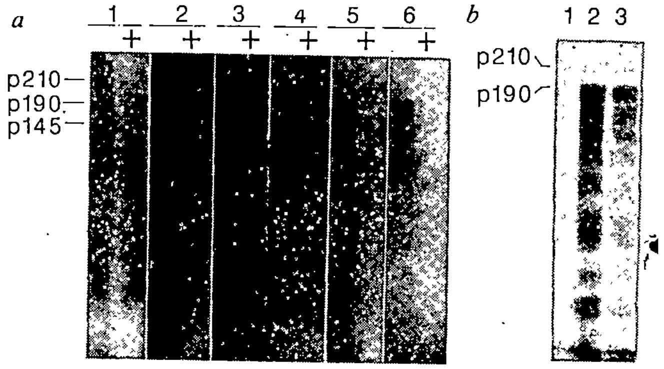

Fig. 2 *In vitro* protein tyrosine kinase assay of *abl* related proteins. *a*, Proteins were immunoprecipitated in the absence of presence (+) of the cognate peptide 4. This peptide sequence is just 3′ to the *abl* protein kinase domain[17]. Protein preparations were isolated from: lane 1, HPB ALL; lane 2, Nalm-1; lane 3, patient F.B.; lane 4, patient F.Y.; lane 5, patient P.N.; lane 6, patient A.E. *b*, Cells from F.B. (lane 1) and A.E. (lane 2) were mixed (lane 3) before kinase analysis. The autophosphorylated proteins were detected by autoradiography.

Methods. Mononuclear cells from clinical samples were isolated on Ficoll-Hypaque (Pharmacia) gradients. Cells ($\sim 10^7$) were lysed in 1 ml of buffer containing 1% Triton X-100, 0.05% SDS, 10 mM sodium phosphate *p*H 7.0, 150 mM NaCl, 5 mM phenylmethylsulphonyl fluoride, 70 $\mu g\,ml^{-1}$ of each of the following proteinase inhibitors: α_2-macroglobulin, bestatin, aprotinin, trypsin inhibitor and leupeptin. The samples were centrifuged for 10 min at 16,000*g* to remove insoluble material and divided into two tubes containing affinity-purified antibody which we have raised against peptide 4 and 30 μl of packed resin bound α_2-macroglubulin (Boehringer-Mannheim). To one tube (+) 0.5 mM of the cognate peptide was added. After 15 min incubation on ice the tubes were again centrifuged and the supernatant transferred to a tube containing 10 μl packed protein A-Sepharose beads. The tubes were mixed gently and allowed to incubate for 20 min on ice. The beads were washed with lysis buffer, without SDS, resuspended in 20 μl of 20 mM PIPES *p*H 7.0, 20 mM $MnCl_2$, and 25 μCi of >5000 Ci $mmol^{-1}$[γ-^{32}P]ATP (Amersham) and incubated for 5 min at 30 °C (ref. 2). The beads were then washed twice with lysis buffer, boiled in 100 μl sample buffer, and electrophoresed through an 8% polyacrylamide gel[18]. The fixed gel was dried and autoradiographed for several hours (lanes 2-6) or overnight (lane 1) at −70 °C using Kodak XAE-5 film.

bcr$^-$ ALL cases or in three other Ph$^-$ cell lines—HPBALL (T cell; Fig. 2*a*); KG1 (myeloid cell) and Nalm-6 (B cell) (data not shown). To exclude the possibility that specific degradation of the p210 to p190[10] occurs in these samples during cell lysis, the *in vitro* kinase assay was repeated using extracts obtained from a mixture of cells from patients F.B. (p210) and A.E. (p190) (Fig. 2*b*). Both p210 and p190 were clearly visible, showing that the novel p190 *abl* protein is unlikely to be a degradation product of p210. In addition, protein extracts from the leukaemia cells from six patients diagnosed as CML in lymphoid blast crisis contained the p210 species (data not shown). If a specific protease were associated with lymphoid blast cells, we would expect a similar observation in this series of samples.

Previously, *in vitro* phosphorylation of p210 has been shown to occur primarily on tyrosine with a trace amount of phosphoserine[10]. Phosphoaminoacid analysis[11] of *in vitro* phosphorylated p190 demonstrated that, as for p210, >95% of the ^{32}P-incorporation occurred on tyrosine residues (data not shown). The p190 appears to have enhanced protein-tyrosine kinase (PTK) activity similar to that of the p210 but because of the way we measure the *abl* protein in an *in vitro* assay we cannot exclude the possibility that the increase we observe in p190 PTK level in the cell might be due to an increase in the *abl* protein level.

We looked for rearrangements in the DNA upstream of the c-*abl* gene. When the DNA blots used for *bcr* analysis were re-hybridized with the 520-bp *Eco*RI fragment localised 17 kb upstream of the v-*abl* homologous coding sequences (designated 0.52E, see ref. 12) (Fig. 3*a*) an additional band was noted in both the *Bgl*II and *Bam*HI digests of patient F.Y. (Fig. 3*b*). It is not surprising that rearrangements were not observed in DNA from the other patients, since Ph$^+$ CML, the break on chromosome 9 band q34 can occur in a region 10 kb to >100 kb upstream[13] of the v-*abl* homologous region of the c-*abl* gene.

The mechanism of activation of c-*abl* p190 PTK in Ph$^+$, bcr$^-$ ALL is unclear. We isolated poly(A)$^+$RNA from frozen leukaemic cells from patient A.E. and compared it to poly(A)$^+$ RNA from KG1 and K562 cell lines on RNA blots. KG1, a myeloid cell line, expresses the normal p145 *abl* protein (data not shown) as well as the normal 6.0 and 7.0 kb *abl* mRNA species (Fig. 4, lane 3). K562 is a cell line derived from a patient diagnosed as CML in blast crisis; it contains the p210[10] and expresses the normal *abl* as well as the 8.7 kb *bcr-abl* hybrid mRNAs (ref. 14 and Fig. 4, lane 1). No abnormal sized transcripts were observed in the RNA from A.E. (Fig. 4, lane 2) although there appears to be more of the 7.0-kb compared to the 6.0-kb mRNA species. In addition, abnormal c-*abl* transcripts have not been detected in two other such cases studied[6,7], but the predicted size of the hybrid c-*abl* transcript could be very similar to the normal c-*abl* mRNA and therefore difficult to resolve by gel electrophoresis.

Our observations suggest that two different molecular mechanisms involving c-*abl* on chromosome 9 and genetic information on chromosome 22 band q11 can generate enhanced kinase activity or result in stabilization or elevated expression of the c-*abl* protein in Ph$^+$ ALL. One involves the *bcr* identified

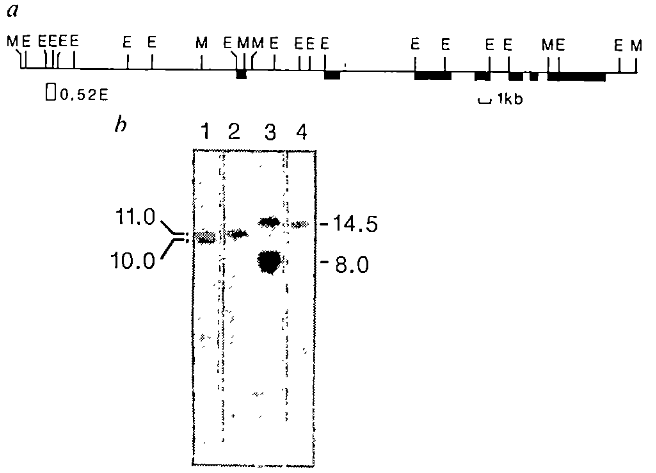

Fig. 3 DNA analysis to detect *abl* rearrangements. *a*, Restriction map for the 5′ region of c-*abl* located on chromosome 9 band q34. Closed boxes, regions homologous to v-*abl*; open box, the probe 0.52E. Abbreviations for enzyme sites are as in Fig. 1*a*. Scale bar, 1 kb. *b*, DNA from F.Y. (lane 1 and 3) or Bri-7 (lanes 2 and 4) was digested with BglII (lanes 1 and 2) or *Bam*HI (lanes 3 and 4) and treated as in Fig. 1*b*. The blot was hybridized to the upstream *abl* probe labelled by the random primer method[19]. Sizes of fragments hybridizing in kb.

originally in Ph$^+$ CML while the other appears to involve sequences outside the designated 5.8-kb *bcr*. Because the latter mechanism has not been detected in a large series of chronic phase Ph$^+$ leukaemia (CML) cases, there appears to be some association between these two mechanisms and disease status. Note that we have not determined whether another region of the *bcr* (or *phl*) gene on chromosome 22, might be involved in this translocation. Interestingly, we have recently screened 13 cases of ALL without the Ph chromosome and identified one which appears to express a normal (p145) sized *abl* related protein with enhanced PTK activity or elevated level of the protein (C. Girdham and K.K.K., data not shown). This may represent a third mechanism for the activation of the c-*abl* proto-oncogene.

It has been suggested that Ph$^+$, bcr$^+$ ALL, which produces an 8.7-kb aberrant *bcr-abl* transcript can be considered to be Ph$^+$ CML in which lymphoid blast crisis has occurred after an undetected chronic phase arising in multipotential progenitor cells[6] (see also refs 4, 5). Our DNA data on patient F.B. which shows rearrangement in the *bcr* and the cellular expression of p210 is consistent with this hypothesis. Ph$^+$, bcr$^-$ ALL might then represent *de novo* B lymphocyte precursor ALL. It is uncertain at present, owing to the small number of cases in our study, whether Ph$^+$, bcr$^-$ ALLs which produce the novel c-*abl* p190 protein are indeed clinically distinct from Ph$^+$, bcr$^+$ ALL. This can perhaps be assessed by testing for c-*abl* p190 PTK in remission and by clinical follow up of remission duration and disease evolution of relapse in a larger series of patients. What is clear is that this group includes adults as well as children, in contrast to previous studies which suggested that Ph$^+$, bcr$^-$ ALL is primarily a childhood disorder[7,15]. A summary of the Ph$^+$ ALL cases in our study is shown in Table 1.

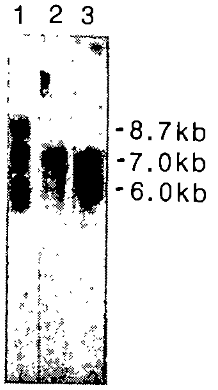

Fig. 4 RNA analysis for altered *abl* mRNA sequences. Poly(A)$^+$ RNAs (6–10 μg) were analysed by electrophoresis, blotting and hybridized to a v-*abl* kinase domain probe. RNAs were purified from K562, a bcr$^+$ cell line derived from a patient with CML in blast crisis (lane 1); cells from patient A.E. (lane 2); and KGl, a myeloid cell line with no Ph involvement (lane 3). RNA sizes in kb as determined from size markers.

Methods. RNA was isolated from cells using the guanidinium thiocyanate method followed by centrifugation through CsCl[20] and enriched for poly(A)$^+$ RNA by oligo-dT chromatography[21]. The RNA was denatured, electrophoresed through a 6% formaldehyde, 0.8% agarose gel[22], transferred to Genescreen Plus (NEN) and immobilized by exposure to a UV light source for 5 min followed by baking for 2 h at 80 °C. The blot was then hybridized to a nick-translated v-*abl* probe[14] and autoradiographed.

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Rapid rotation of flagellar bundles in swimming bacteria

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A bacterial flagellum is driven by a reversible rotary motor[1–3]. The power input is determined by protonmotive force and proton flux, the power output by torque and speed: interrelationships between these parameters provide important clues to motor mechanisms. Here we describe the relationship between torque and speed at constant protonmotive force. The measurements are analogous to those that could be made by plugging an electric motor into a constant-voltage outlet, varying the external load, and determining the torque delivered at different speeds. We used suspensions of metabolizing cells of a motile *Streptococcus*, varied the external load by changing the viscosity of the medium, determined motor speed from the frequency of vibration of the cell body, and inferred motor torque from the rate of body rotation. The flagellar bundles rotate more rapidly than formerly supposed, at rates that increase linearly with temperature. The torque delivered by the flagellar motor drops linearly with speed. At high speed, the torque-generating cycle associated with the transfer of one proton appears to dissipate free energy in a series of small steps.

The helical bundle of a swimming bacterium generates thrust, and the cell translates at a velocity at which this force is balanced

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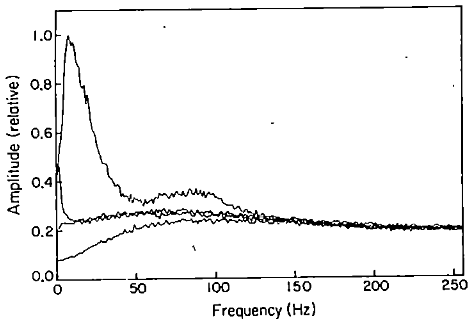

Fig. 1 Amplitude spectra of swimming cells of *Streptococcus* (top curve), of de-energized cells moving at the speed of the swimming cells (second curve), of de-energized cells undergoing Brownian movement (third curve), and of the background illumination at the same total light intensity (bottom curve).

Methods. Cells of a smooth-swimming mutant, SM197, were grown at 35 °C in KTY medium^2, collected at late exponential phase, washed twice by centrifugation (480g, 6 min) in an equal volume of 0.1 M sodium phosphate (*p*H 7.5), 0.2 M KCl, 0.1 mM EDTA, 0.01 M D-glucose, and then resuspended in this medium at a density of $\sim 4\times10^8$ cells ml^{-1}. An aliquot of this suspension was drawn into a flow chamber^{22} (0.4 mm deep) and examined at 22 °C under an inverse phase contrast microscope (see below), yielding the spectrum shown in the top curve (mean swimming speed ± s.e.m., 15.7 ± 1.1 μm s^{-1} for 17 cells, as judged from slow-speed playback of a video recording^{23}). Another aliquot was de-energized by the addition of the uncoupler FCCP (trifluoromethoxycarbonylcyanide phenylhydrazone, 10 μg ml^{-1}) and either drawn through the chamber at a speed of 14.4 μm s^{-1} (measured at the middle of the chamber), yielding the spectrum shown in the second curve, or examined when stationary, yielding the spectrum shown in the third curve. The microscope (Nikon S-Ke) was equipped with a 100 W tungsten-halogen lamp (type FCR, driven by a constant-voltage d.c. power supply), a heat-transmitting mirror (Optical Industries 03MCS007), a CF BM ×40 objective, a ×20 eyepiece, a $\frac{1}{2}\times$ PFM photomicrographic attachment, and a photomultiplier tube (RCA 4886, run at 300 V). The focal plane was located midway between the top and bottom windows of the flow chamber to minimize wall effects. The diameter of the photocathode (1.8 cm), referred to the focal plane, was ~39 μm. The output of the photomultiplier was passed through a current-to-voltage converter (10^7 Ω feedback), a difference amplifier (to remove the d.c. offset and amplify the a.c. signal), a double-pole low-pass filter, and a single-pole high-pass filter. The 3 dB cutoff points of these filters were set at 200 and 72 Hz, respectively. A 12-bit analogue-to-digital converter sampled data at 512 points s^{-1} for one-second intervals and a PDP-11/34 computer computed and averaged the corresponding power spectra (400 times for the data shown). The signals arose from lateral motion of images on the photocathode, which was exposed to light from only a small region of the flow chamber, and which when scanned with the image of a cell that was stuck to glass, was found to have an output (signal less background) that rose and fell 3 or 4 times across a diameter, varying in amplitude by as much as 50%. Contributions from out-of-focus components of the images were significant, as useful spectra could be obtained on moving the plane of focus beyond the limits of the flow chamber. The basic features of the spectra, including sidebands due to non-linearities encountered with larger displacements (see text), could be reproduced by mounting the detector on the tracking microscope^{24}, suspending de-energized cells in the tracking chamber, and moving it sinusoidally with the tracker drive. Much larger signals were obtained on moving the tracking chamber sideways than up and down. De-energized cells and monodisperse polystyrene latex spheres (1.3 μm diameter) gave identical spectra.

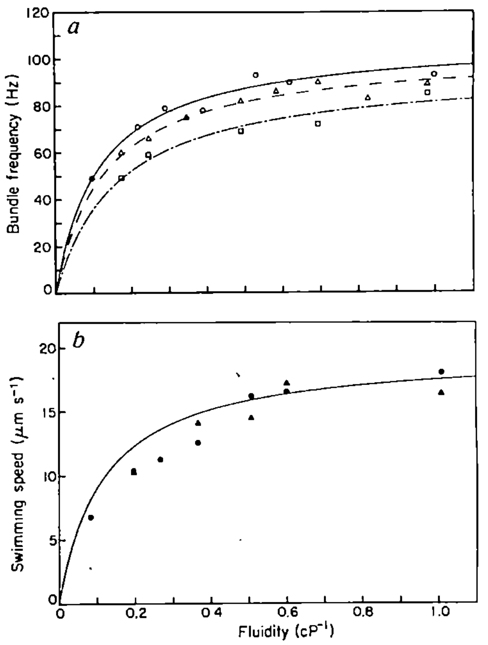

Fig. 2 Bundle frequencies (*a*) and swimming speeds (*b*) of *Streptococcus* as a function of the fluidity of the medium. The different symbols refer to cells from different cultures. The values shown at each point are means for the cell population determined from the power spectrum or the tracking data, respectively. In either case, the spread (s.d. divided by the mean) was about 25%. The fits to the data in *a* are shown in Fig. 3. The solid curve in *b* is the solid curve in *a*, redrawn and scaled to match approximately the swimming speeds at high fluidity.

Methods. To measure bundle frequencies, cells of strain SM197 were grown, collected, washed as described in Fig. 1. Then (open circles) cells were resuspended at 80 times the density at collection. An aliquot was mixed with 9 vols of Ficoll 400 solution of known concentration. Alternatively (open triangles and open squares), cells were pelleted once, as before, and resuspended in a solution of Ficoll in KTY medium. All spectra were taken as described in Fig. 1, except in a shallower chamber (0.15 mm deep)^{23}. Ficoll standards were prepared by serial dilution of a 20% w/v stock prepared in the medium in which the cells were suspended. The viscosities of these solutions were measured at 22 °C in a Cannon-Ubbelohde viscometer (viscometer constant 8.13×10^5 cm^2 s^{-2}). The same stock was used to prepare all the Ficoll solutions. The position of the centre of the high-frequency peak was found as follows. The power spectrum for non-motile cells was smoothed with a cubic spline procedure^{25} and subtracted from the power spectrum for the swimming cells. The difference spectrum was smoothed in the same way, and its low-frequency peak was fitted by a curve generated by computer simulation (see text). This curve was subtracted out, and the centre of the remaining (high-frequency) peak was found by eye. To measure swimming speeds, cells of the wild-type strain V4051 (ref. 26) were grown, collected and washed as described in Fig. 1, and then resuspended at 2.5 times the density at collection. An aliquot was mixed with 50 volumes of a solution of Ficoll of known concentration, and the swimming speeds of 20–30 cells were found by tracking^{24}. The following data (mean ±s.d., unless otherwise noted) were obtained for a population of 60 bacteria in the absence of Ficoll (compare ref. 27, Table 1): speed 16.8 ± 3.7 μm s^{-1} (mean ±s.e.m.), tumble length 0.18 ± 0.07 s, run length 1.71 ± 0.90 s, change in direction from run to run 63±14°, and change in direction during runs 26±8°. The run-tumble statistics also were Poisson.

by viscous drag on the body; the bundle also generates torque, and the body counter-rotates (or rolls). If the longitudinal axes of the bundle and the cell body are not collinear, then the image of the body wobbles from side to side. If there is a net imbalance in the forces perpendicular to the axis of the helix, for example, if the bundle does not contain an integral number of wavelengths^4, then the cell body also vibrates (or gyrates) at the rotation frequency of the bundle. The presence of such motion was postulated long ago by Reichert^5, who referred to it as *di* *Trichterbewegung*, or funnel movement^6.

We measured these frequencies by projecting the images o swimming cells onto the photocathode of a photomultiplier tub whose sensitivity is spatially inhomogenous. The output wa passed through a bandpass filter and the power spectral densit was computed with the fast Fourier transform. The spectra o many successive one-second data blocks were averaged. Th

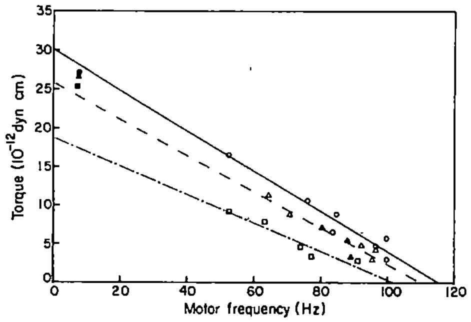

Fig. 3 Torque of the flagellar motor of *Streptococcus* as a function of speed. The open symbols are values derived from the data of Fig. 2*a*. The lines are least-squares fits to these data. The closed symbols are values derived from experiments on cells tethered by a flagellar filament[11].

Methods. For swimming cells, the torque exerted by the bundle on the cell body is given by the product of the body-roll frequency, the viscosity of the medium, and a geometrical factor that depends on the shape of the cell and its axis of rotation. We approximated the cell body as a cylinder of length 3.27 μm and width 1.27 μm rolling about an axis that intersects the cylinder axis 1.09 μm from one end of the cell at an angle of 22°. Cell sizes were determined from photomicrographs of cells that had settled on a glass surface, whereas other parameters related to the geometry were estimated by inspecting the trajectory of several cells on the video record. The corresponding drag coefficient was computed from published formulae[28,29]. No correction was made for the additional viscous drag on the body due to the counter-rotation of the flagellar bundle or for loss of torque due to interactions of flagella within the bundle; the latter assumption is justified by the observation that free hooks[2] or flagellar stubs[7] spin at rates comparable to those of flagellar bundles. We visualized the flagellar filaments with a modified Ryu stain[30] and found an average of 3.5±0.2 flagella per cell, all of which were assumed to join in a single bundle. For tethered cells, cells from three cultures were prepared as described in Fig. 1 and tethered to a silanized cover slip[11]. We approximated the cell body as a cylinder of length 3.04 μm and width 1.36 μm spinning about an axis perpendicular to the cylinder axis 0.32 μm away from the centre of the cell. No correction was made for the additional viscous drag due to the proximity of the cover slip[31].

top curve of Fig. 1 shows the amplitude of this spectrum (the square-root of the power spectrum) obtained from a field of smooth-swimming cells of *Streptococcus*; the low-frequency peak is due to the roll of the cell body and the high-frequency peak to its funnel movement. The other spectra in Fig. 1 characterize noise arising from changes in the number of cells imaged on the detector, Brownian movement and photoelectron statistics.

To interpret these spectra better, we simulated the measurements by computer, moving a vibrating spot across a detector with a radial gaussian sensitivity and filtering the output. We concluded from this analysis that the low-frequency peak (Fig. 1) is skewed toward high frequencies because of harmonics of the body-roll frequency that arise from the nonlinear relation between displacement and detector output at large wobble amplitudes. The high-frequency peak, on the other hand, although broadened somewhat by sidebands generated by mixing with the low-frequency signal, remains centred at the mean bundle frequency, provided (as in Fig. 1) that the two peaks do not considerably overlap. Both peaks are broadened and smoothed by variations over the cell population[7].

The correct body-roll frequency corresponding to each amplitude spectrum was determined by examining video recordings made of the same cell suspensions. An average was computed from the wobble frequencies of all the cells in a video field, as measured by eye and stopwatch during slow-speed playback. For the culture used in the experiments of Fig. 1, the body-roll frequency was 6.7±2.4 Hz (mean ±s.d. for 20 cells). Given a bundle frequency of about 95 Hz (Fig. 1), this yields a value for the speed of the flagellar motor relative to the cell body of about 100 Hz.

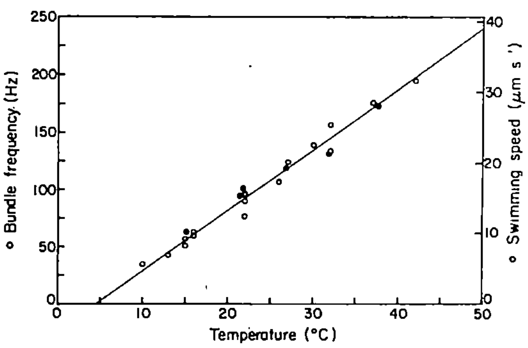

Fig. 4 Bundle frequencies (○) and swimming speeds (●) of *Streptococcus* as a function of temperature. The values shown are the means for the cell population, determined at each point from the power spectrum or the tracking data, respectively. In either case, the spread was about 25%. The temperature was controlled as described previously[10]. Other conditions were as in Fig. 2 (with cells prepared in phosphate buffer). The line is a least-squares fit to the bundle-frequency data. The swimming-speed scale was adjusted so that the swimming-speed data fell along the same line.

Figure 2 shows the dependence of bundle frequency and swimming speed on the fluidity of the medium, adjusted by addition of Ficoll, a highly branched viscous agent whose solutions show newtonian properties[8]. A tenfold increase in viscosity was required to halve the bundle frequency. Swimming speeds were closely correlated with bundle frequencies, but they were slightly lower at higher viscosities: the ratio of swimming speed to bundle frequency decreased from 0.18±0.01 μm to 0.15±0.01 μm for the data shown. A possible explanation for this trend is that the bundle winds up slightly as the torque increases, decreasing its propulsive efficiency. We also measured bundle frequencies of *Streptococcus* as a function of fluidity using methylcellulose and found that the frequencies decreased monotonically with increasing viscosity (data not shown). Thus, increases in swimming speed observed on the addition of small amounts of this agent[9] must arise from enhancement of the efficiency of flagellar propulsion, as argued previously[8], not from changes in the speed of the flagellar motors.

Figure 3 shows the torque generated by the flagellar motor as a function of its rotation rate. The open symbols are a different representation of the data of Fig. 2*a*: their position on the abscissa is the sum of the body-roll and bundle frequencies, equal to the motor rotation frequency, whereas their position on the ordinate is proportional to the product of the body-roll frequency and the viscosity of the medium. The proportionality constant is determined by a geometrical drag factor and the number of filaments per bundle. The same constant was assumed for all the experiments on swimming cells. For the three cultures, the torque appears to drop linearly with increasing speed, although the relationship varies somewhat from culture to culture. The closed symbols represent the torque for tethered cells, calculated in the same fashion, but with a different geometrical drag factor. These torques correspond well to the linear extrapolation of the data for swimming cells. Note that the ratio of the two drag factors, and thus the position on the ordinate of the closed symbols relative to the open symbols, is subject to considerable uncertainty, probably up to 40%. The implications of Fig. 3 for the mechanism of torque generation are discussed below.

Figure 4 shows the dependence of bundle frequency (open symbols) and swimming speed (closed symbols) of *Streptococcus* on temperature. Both increase linearly over the range 10 to 40 °C. To check for a possible temperature dependence of the protonmotive force, we tethered cells from the culture used for the bundle-frequency measurements. Their torques were compared at 9.3, 16.0, 22.0, 27.8 and 31.6 °C and found to fall in the ratios 0.7, 0.95, 1.00, 1.02 and 1.04, respectively (torques for each of 14 cells were scaled to the torque at 22 °C and then averaged; the standard deviation for the population was ~0.05; the mean speed at 22 °C was 6.3 Hz). Artificially energized cells generate a torque that is essentially independent of temperature over the range 4-38 °C[10]. Assuming a linear dependence of torque on protonmotive force[10,11], we conclude that the protonmotive force of metabolizing cells increases by at most 10% over the temperature range 16-32 °C. Over this range, the bundle frequencies of the swimming cells increased by a factor of 2.4 (Fig. 4). A linear dependence of swimming speed on temperature has been observed for *Escherichia coli* using number-fluctuation spectroscopy[12] and cinematography[13]. Similar results were obtained for *Salmonella typhimurium* by recording motility tracks[14].

We also measured the bundle frequencies and swimming speeds of *E. coli*. The mean bundle frequency of a smooth-swimming strain (HCB437) grown at 35 °C on glycerol in a minimal salts medium, collected at mid-exponential phase, and then studied in motility medium[15] at 22 °C—these were the conditions used for the measurements of rotational frequencies of hooks described earlier (mean ± s.d. for 20 cells 104 ± 29 Hz)[2]—gave a result in the same range, 113 Hz. The mean bundle frequency for these cells was higher at 32 °C, 156 Hz. We also determined mean bundle frequencies for cells grown at 35 °C in tryptone broth and collected at mid-exponential phase. These frequencies were higher still, 191 Hz at 22 °C, and 268 Hz at 32 °C. All these measurements were made near the open edge of the chamber[8] to ensure that the cells were well oxygenated. Cells of wild-type strain AW405 (ref. 16) were grown on tryptone broth and tracked at 32 °C in motility medium containing varying amounts of Ficoll. The dependence of swimming speed on fluidity was essentially identical to that observed for *Streptococcus* (Fig. 2*b*), except that the swimming speeds were higher (mean ±s.e.m. for 79 cells at fluidity 1.25 cP^{-1}, 36.4 ± 1.0 $\mu\mathrm{m\,s}^{-1}$).

What can these measurements tell us about the physics of flagellar propulsion? The torque of the motor drops monotonically with increasing speed, and the relationship appears to be approximately linear over a wide range (Fig. 3). These results do not favour a mechanism by which the motor runs at constant power: in that case the torque would be inversely proportional to speed, and the fits would be hyperbolic. This conclusion is reinforced by two additional observations, namely, that the torque generated by a tethered cell is approximately constant for speeds from ~10 Hz to stall (ref. 11 and M.M., H.C.B., in preparation), and that the speed observed with hooks approximates the speed observed with flagellar bundles. The frictional drag coefficient of a hook is very much smaller than that of a filament in a bundle. Therefore, the intercepts of the linear fits with the axes of Fig. 3 are confirmed experimentally.

In the framework of thermodynamics, one can treat the flagellar motor as a system coupling two flows: the flux of protons and the rotation of the rotor. If the system operates close to thermodynamic equilibrium, then the flows are linear functions of the pair of conjugate driving forces, namely, the protonmotive force and the torque acting on the rotor[17]. When the protonmotive force is held constant, as it was in the measurements of Fig. 3, torque should decrease linearly with speed. Chemical processes occur close to equilibrium when the difference in the free energies of reactants and products (dissipated as heat) is small compared to kT, where k is the Boltzmann constant and T is the absolute temperature. The observed linear relationship between torque and speed appears to extend to the point where the motor generates essentially no torque and, thus, performs no mechanical work. At this speed, all the free energy available per proton (~8 kT) is dissipated by processes internal to the motor. The motor could still operate in the linear regime if the torque-generating cycle associated with the transfer of one proton involved a series of many reactions, each of which entailed only a small free energy loss.

A mechanism that has this feature has been proposed by Läuger[18], where protons are translocated from the external medium into the cytoplasm along binding sites at the intersections between rows of ligands on the faces of the M and S rings. The two sets of rows are tilted with respect to each other, so proton movement is coupled to motor rotation. Läuger discussed the operation of this device at low speeds and suggested that the torque should be independent of speed below 10 Hz. At the much higher speeds observed in swimming cells, finite proton transfer rates might lead to considerable dissipation of free energy conduction along the ligand chain and thus limit the torque. If each row contains 10 or more sites, the torque might still vary linearly with speed.

In a model proposed by Berg and Khan[19], a single torque-generating step uses all the electrochemical energy of a proton and stores it as elastic energy in a spring. This process becomes rate-limiting at high speeds and accounts for all the internal dissipation. A preliminary analysis of this mechanism at steady state predicts a nonlinear dependence of torque on speed that is inconsistent with the data of Fig. 3. However, the model can be altered successfully by assuming that protons reach the rotor by stepping along a series of sites in a membrane channel, provided that the latter process is rate-limiting at high speeds.

An approximately linear torque-speed relationship can be obtained from mechanisms that work far from thermodynamic equilibrium, but only explicit construction. An example is the model proposed by Oosawa and Hayashi[20], in which proton transfer and rotation are loosely coupled. Measurements of torque at high speed place strict requirements on all models for the bacterial motor and deserve more attention.

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Mammalian and bacterial sugar transport proteins are homologous

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The uptake of a sugar across the boundary membrane is a primary event in the nutrition of most cells, but the hydrophobic nature of the transport proteins involved makes them difficult to characterize. Their amino-acid sequences can, however, be determined by cloning and sequencing the corresponding gene (or complementary DNA). We have determined the sequences of the arabinose-H$^+$ and xylose-H$^+$ membrane transport proteins of *Escherichia coli*. They are homologous with each other and, unexpectedly, with the glucose transporters of human hepatoma[1] and rat brain[2] cells. All four proteins share similarities with the *E. coli* citrate transporter[18]. Comparisons of their sequences and hydropathic profiles yield insights into their structure, functionally important residues and possible evolutionary relationships. There is little apparent homology with the lactose-H$^+$ (LacY)[3] or melibiose-Na$^+$ (MelB)[4] transport proteins of *E. coli*.

In the bacterium *Escherichia coli* there is one H$^+$-linked transport system for arabinose encoded by the gene *araE*[5], and another for xylose encoded by *xylE*[6] (with gene products AraE and XylE). The linkage of sugar and H$^+$ translocation enables energization of nutrient uptake by the transmembrane electrochemical proton gradient[7,8].

We have isolated and sequenced the *araE* and *xylE* genes, and deduced the amino-acid sequence of each protein (Fig. 1). AraE contains 472 amino acids (relative molecular mass (M_r)51,683), and XylE contains 491 amino acids (M_r 53,607). Both are typical membrane proteins: they are very hydrophobic (about 67% nonpolar residues); their hydrophobic and hydrophilic regions are arranged alternately (Fig. 1; see also ref. 9); and their calculated M_r values are substantially higher than the values of ~36,000 for AraE[8] and 39,000 for XylE[8] measured by SDS-polyacrylamide gel electrophoresis.

The amino-acid sequence of the glucose transport protein, which does not translocate a cation, from a human hepatoma cell line (HepG2) was recently determined[1]. The reading frame

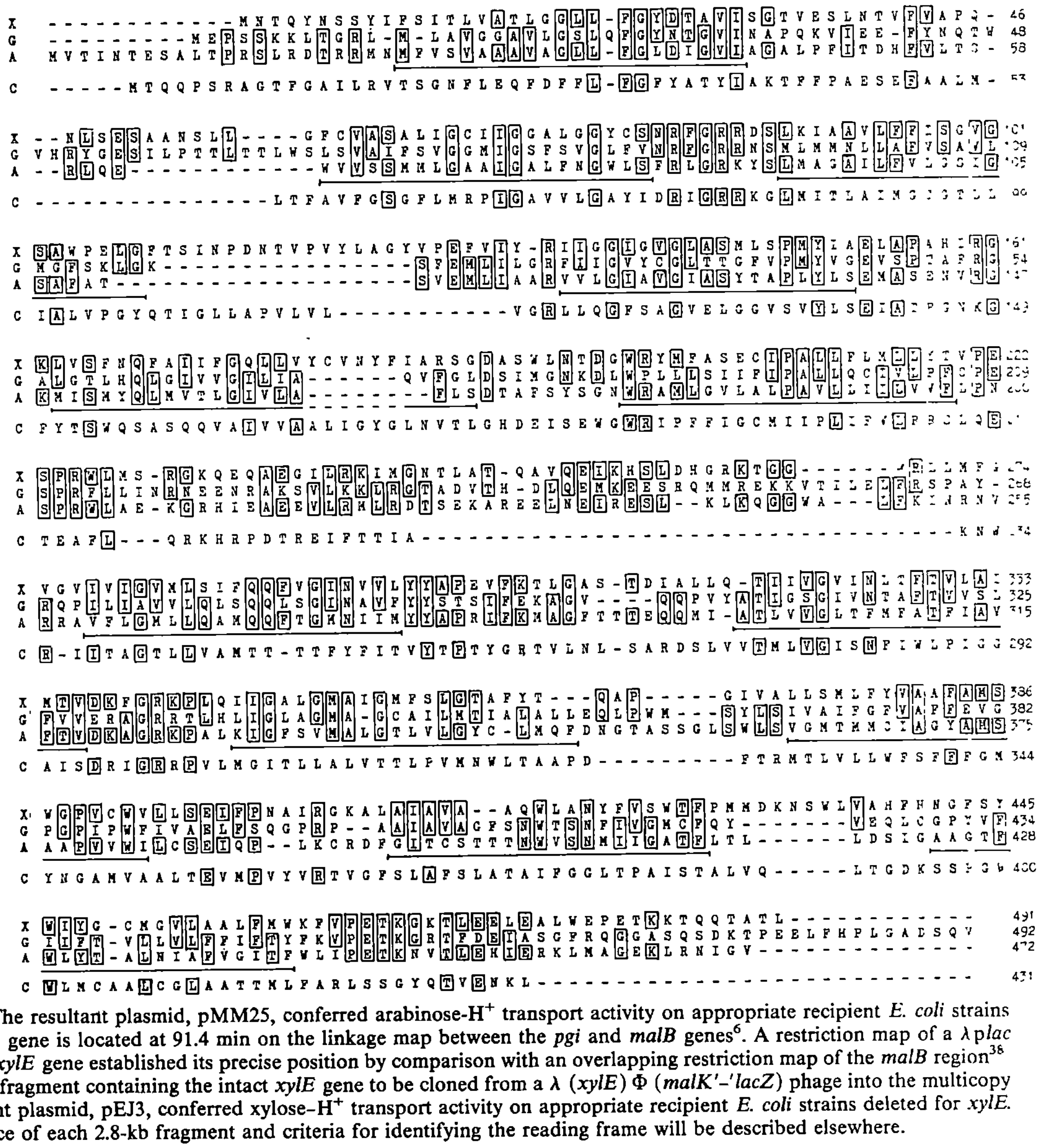

Fig. 1 Aligned sequences of the *E. coli* xylose-H$^+$, arabinose-H$^+$ and citrate transporters with the human hepatoma glucose transporter. The sequences of the glucose and citrate transport proteins were taken from refs 1 and 18, respectively. Residues are boxed in the XylE, glucose and AraE transporters if they occur more than once at an aligned position in the three sugar transporter sequences. They are only boxed in the citrate transporter if they also occur in the aligned positions of two or three of the sugar transporters. The predicted membrane-spanning regions of the glucose transporter[1] are underlined. X, XylE; A, AraE; C, citrate transporter; G, glucose transporter.

Methods. The *araE* gene is located at 61 min on the linkage map close to the *lysA* gene[8]. A restriction map of a λ*plac* Mu phage inserted into the *araE* gene established its precise position by comparison with an overlapping restriction map of the *lysA* region[36]. This enabled a 2.8-kilobase (kb) DNA fragment containing the intact *araE* gene and promoter region to be cloned from a specialized λ transducing phage[37] into the multicopy vector pBR322. The resultant plasmid, pMM25, conferred arabinose-H$^+$ transport activity on appropriate recipient *E. coli* strains negative for *araE*. The *xylE* gene is located at 91.4 min on the linkage map between the *pgi* and *malB* genes[6]. A restriction map of a λ*plac* Mu phage inserted into the *xylE* gene established its precise position by comparison with an overlapping restriction map of the *malB* region[38]. This enabled a 2.8-kb DNA fragment containing the intact *xylE* gene to be cloned from a λ (*xylE*) Φ (*malK′-′lacZ*) phage into the multicopy vector pBR328. The resultant plasmid, pEJ3, conferred xylose-H$^+$ transport activity on appropriate recipient *E. coli* strains deleted for *xylE*. The complete DNA sequence of each 2.8-kb fragment and criteria for identifying the reading frame will be described elsewhere.

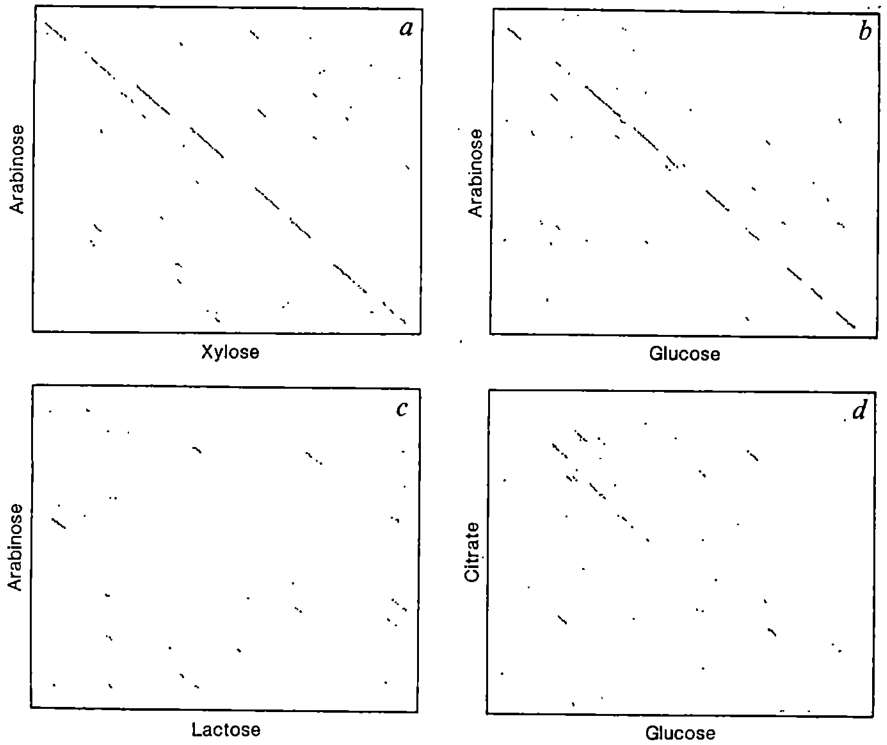

Fig. 2 Comparisons of the sequences of transport proteins using Diagon plots[10]. *a*, The arabinose-H$^+$ and xylose-H$^+$ transport proteins of *E. coli*; *b*, the arabinose-H$^+$ transport protein of *E. coli* and glucose transport protein of human hepatoma cells; *c*, the arabinose-H$^+$ and lactose-H$^+$ transport proteins of *E. coli*; *d*, the citrate transport protein of *E. coli* and the glucose transport protein of human hepatoma cells. The comparisons used a 21-residue segment and generated a dot if the score exceeded 240 (ref. 10).

was confirmed by the identity of parts of its sequence with peptides derived from the erythrocyte glucose transporter[1]. Its amino-acid composition was similar to that of the AraE and XylE proteins with 492 amino acids (M_r 54,117)[1]. The sequence of the glucose transporter from rat brain cells, identified and cloned using similar techniques, was virtually identical, having 492 amino acids (M_r 56,133)[2].

The sugar-transporter sequences were compared using the Diagon algorithm of Staden[10]. Several homologous regions were revealed (Fig. 2*a*, *b*) in both hydrophobic and hydrophilic regions (Fig. 1). Furthermore, their hydropathic profiles[9,11] were similar (Fig. 3); there was an hydrophilic segment, 50–65 residues long, located approximately at the mid-points of each of the sequences (Fig. 3), with some similar hydrophilic segments either side marked X in Fig. 3, and a similar number (12) of hydrophobic segments (Fig. 3).

Diagon plots drawn at the same and less stringent levels of discrimination did not reveal such homologies between XylE, AraE or the glucose transporter and the lactose[3] or melibiose[4] transporters of *E. coli* (one example is shown in Fig. 2*c*). It should be noted that the scoring matrix used by the Diagon algorithm was not devised for membrane proteins.

The sequence of the passive glucose transporter[1] is aligned with the sequences of the active arabinose and xylose transport proteins in Fig. 1. Seventy-seven residues are conserved in all three proteins (the glucose transporter has 131 identitites with AraE and 142 identities with XylE; AraE and XylE have 141 residues in common). There are additional conservative substitutions[12] throughout the sequences. Consequently nearly 40% of residues can be regarded as homologous, a level sufficient to expect similar secondary and tertiary structures for all three proteins. Like many integral membrane proteins[13] these transporters lack the N-terminal signal sequences typically required for insertion of proteins through membranes[14]. The attachment site for the carbohydrate chain, Asn 45 of the hepatoma cell glucose transporter[1,15], is not conserved in the XylE or AraE proteins (Fig. 1).

The citrate-H$^+$ transporter[16] of *E. coli* has 431 amino acids (M_r 46,979)[17,18] and an apparent M_r of 35,000[19] (determined by SDS-polyacrylamide gel electrophoresis. Diagon comparisons revealed little homology with AraE or XylE, but some homology with the glucose transporter (Fig. 2*d*). However, all four proteins had similar hydropathic profiles (Fig. 3) with 12 membrane-spanning regions predicted by the Eisenberg algorithm[11] (Fig. 1, Fig. 3). This enabled us to align the patterns of conserved amino acids in all four transporters, particularly in the regions 30–49, 60–101, 121–149, 257–302 (Fig. 1). These patterns may be coincidental[20], but, more probably, they indicate that those residues boxed in the citrate transporter (Fig. 1) are critical for the common transport function of all the proteins.

It is interesting to locate certain conserved amino acids, in view of previous suggestions as to their roles in transport. For example, a glutamate (or aspartate) residue is responsible for H$^+$-translocation by subunit c in the F$_0$ moiety of H$^+$-ATPase[21], and Glu 325 is implicated in H$^+$-translocation by LacY[22], so the conservation of such residues at positions corresponding to 153, 337, 397 and 472 of XylE (Fig. 1) identifies putative H$^+$-translocating residues in these proteins. The mammalian transporters are not thought to translocate cations, however[23].

There is no conservation of histidines and cysteines (Fig. 1), residues implicated in the transport function of LacY[24–27]. Hence they are unlikely to have a common role in these proteins. In all three sugar transporters substrate(s) protect a thiol group against reaction with *N*-ethylmaleimide[8,28,29], a phenomenon well established in LacY[23,24,30]. As all cysteines in AraE occur between residues 343–400, at least a part of this region of this protein is adjacent to a substrate binding site.

A compelling conserved feature is the motif RXGRR, which is duplicated in each of the four proteins (Fig. 1; Fig. 3 marked X; R may be replaced by K), and occurs in a similar form in LacY and MelB (Fig. 3). It is predicted by the Robson algorithm[31] to form a β-bend in six of its eight locations. This may link two helices in a defined topology stabilized by charge-charge interactions with head groups of the lipids. Its sequence also resembles peptides recognized by mammalian cyclic AMP protein kinase[32,33].

The central hydrophilic regions of all four proteins are predicted by the Robson algorithm[31] to be α-helices, which correlates with the observation that 20% of the homologous erythrocyte glucose transporter forms non-membrane α-helix[34]. There are

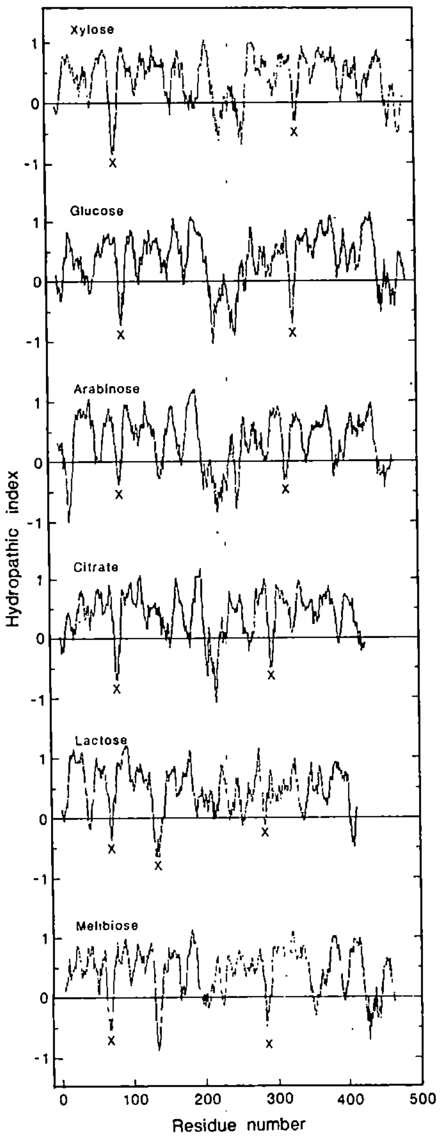

Fig. 3 Hydropathic profiles of transport proteins. Hydropathy values[9] for a window of nine amino-acid residues were averaged and plotted according to the position of the middle residue along the length of each sequence. The sequences of the indicated proteins were taken from Fig. 1 and refs 1, 18, 3 and 4. X indicates the positions of the –RXGRR– motif in each protein.

indications, such as the duplicated RXGRR motif, that the N-terminal and C-terminal halves are arranged symmetrically about this region, with six corresponding membrane α-helices on each side. An internal gene duplication event[20] is thus implied in an ancestral transporter, which then evolved to the present proteins.

The similarity between the sugar transporters from such divergent organisms as bacteria and mammals is probably too great to have arisen by convergent evolution. Gene transfer from eukaryotes to their prokaryotic symbionts remains an explanation: an example of this process has recently been described[35]. But we think it more likely that the homologies reflect functionally important parts of an ancient sugar transporter present in organisms before their divergence into prokaryotes and eukaryotes. If this is the case, homologous sugar transporters may also be found in plants, unicellular eukaryotes and other organisms. Whatever its origin, the similarity of these transport proteins suggests that a combination of biochemical, immunological and genetic techniques can now be used to exploit the advantages of working with prokaryotes to illuminate transport processes in higher organisms.

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Molecular dynamics studied by analysis of the X-ray diffuse scattering from lysozyme crystals

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It is now well established that the biological activity of proteins is related not only to their mean molecular structure, but also to their intramolecular mobility[1–4]. Nearly all techniques sensitive to dynamics have given evidence for intramolecular mobility in proteins: NMR[5,6], ESR[7], Raman spectroscopy[8,9], fluorescence quenching[10], Mössbauer spectroscopy[11], neutron scattering[12], measurements of elastic constants[13] and hydrogen–deuterium exchange[14]. The dynamics of proteins has also been approached by theoretical calculations[15,16]. We report here investigations of the atomic and molecular displacements in hen egg-white lysozyme crystals using a new technique. This technique, based on the X-ray diffuse scattering analysis (scattering out of the Bragg reflections),

can yield information on the atomic displacements, provided that they are correlated. Rigid-body molecular displacements, correlated along short rows of aligned molecules in two perpendicular directions, have been detected and analysed (mean square amplitude of the order of 5×10^{-4} nm^2). This technique can be also applied to the detection and analysis of intramolecular displacements.

Molecular mobility can alter the periodic order of protein crystals, giving imperfect crystals. The concepts of X-ray scattering by imperfect crystals have been clearly explained by Guiner[17] and Cowley[18]. Basically, the effect of imperfections is to split the scattered intensity into two components I_B and I_D. The term I_B corresponds to the set of Bragg reflections; Debye[19] has shown that for small amplitude and harmonic displacements I_B can be derived from the expression which is valid for a perfect crystal by multiplying each atomic scattering factor by the factor exp $(-B|\mathbf{S}|^2/4)$ where $\mathbf{S}$ is the scattering vector and B the temperature factor. For each atom j, $B=8\pi^2\langle \mathbf{u}_s^{j2}\rangle$ where $\mathbf{u}_s^j$ is the projection of the displacement of atom j along $\mathbf{S}$. The term I_D is the diffuse scattering intensity. In contrast to I_B, which depends on the mean value of the scattering function per unit-cell, I_D depends on the local deviations of this function from its mean value and on its spatial correlations; it is spread all over reciprocal space and unlike I_B is not restricted to the reciprocal lattice nodes. Thus, even in the case of long range correlations, I_D will be ≥ 100 times weaker than I_B. Consequently, its observation requires high-intensity beams, long exposures and a low background. Analyses of B-factors and comparison between crystalline structures (which are determined from I_B) has provided valuable information concerning the flexibility of various proteins[20-23].

But more precise B-factor analyses[24] remain limited by various factors. Firstly the $\langle \mathbf{u}^2\rangle$ determinations hold only for small harmonic displacements and values of $\langle \mathbf{u}^2\rangle$ are considered as isotropic for proteins (except in refs 25 and 26). The most important limitation stems from the fact that the thermal factors are individual factors, summing up all the types of displacements, and thus no correlation between the atomic displacements can be derived from their values. The correlation information can be obtained precisely by the diffuse scattering study, which gives access to the deviations from the ideal mean crystalline structure. The existence of diffuse scattering was predicted as early as 1923 by Waller[27], and has been widely and succesfully used in physical and chemical crystallography. This technique has only been used once for proteins by Phillips *et al.*, who derived a model of filament motion for tropomyosin[28]. Our aim is to extend the use of diffuse scattering analysis to biological macromolecular compounds.

We have tested the feasibility of diffuse scattering studies of proteins on hen egg-white lysozyme crystals. The dynamics of this enzyme has been extensively studied by various techniques[9,13,14,16,29-31] and by analysis of thermal factors[23,32]. This work is restricted to the orthorhombic form of lysozyme which is stable at physiological temperatures. The structure of this crystalline form has been determined at 0.2 nm resolution by Berthou *et al.*[33]. The data collection of diffuse scattering has been performed at the Laboratoire d'Utilisation du Rayonnement Electromagnétique (LURE) using synchrotron radiation. Because of the much longer exposure times than for recording Bragg reflections (10^2-10^4 times), only a set of 'still' photographs has been obtained.

On the film, the diffuse intensity can be observed along rows, with a modulated intensity, and is in fact, in the reciprocal space, mainly located along two families of planes: (100)* (perpendicular to a*) and (001)* (perpendicular to c*). Other tiny diffuse features are found along the reciprocal planes (101)* and $(10\bar{1})^*$, but we will not consider these two poorly defined features.

We can at once discard the hypothesis that attributes the diffuse scattering to radiation damage effects, because fresh crystals and old ones display exactly the same diffuse pattern. Because of the well-defined location of I_D, we have used a two-step analysis procedure: determination of the type of correlation involved in the atomic displacements (from I_D location) followed by determination of displacement parameters (from I_D intensity). This type of correlation is simply given by the Fourier transform of equidistant reciprocal planes (distance d^*) which lie in a periodic row (period $1/d^*$) perpendicular to the planes. In other words, the diffuse planes imply the existence in the crystal of periodic finite rows parallel to **a** and **c**, of period $|\mathbf{a}|$ and $|\mathbf{c}|$, which scatter the X-photons in a partially incoherent way because of atomic displacements. More precisely at any given instant, all the equivalent atoms (translation **a** or **c** related atoms) along a row are displaced from their mean position by the same vector **u**, the displacements being uncorrelated between different rows. The average length of these rows is given by the inverse of the thickness (FWMH) of the diffuse planes, (4±1)

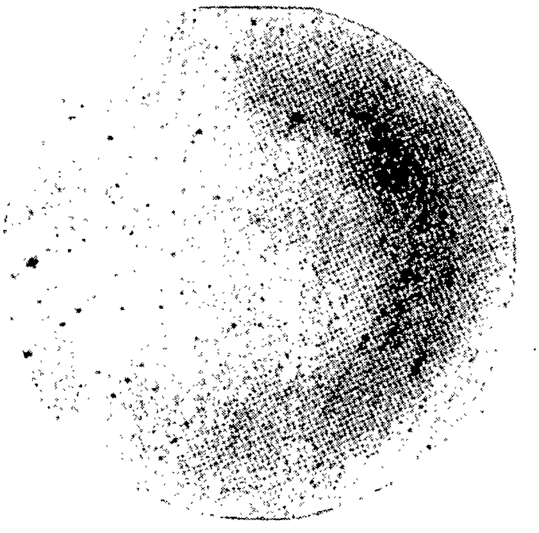

Fig. 1 Typical example of an X-ray scattering pattern from hen egg-white lysozyme crystals (form B). The unit cell is orthorhombic (symmetry $P2_12_12_1$) with the parameters: $a=5.64$ nm, $b=7.38$ nm, $c=3.05$ nm. The crystals were grown at 40 °C from a 5% protein solution buffered at pH 4.7 with 2.0 M acetate to which a 10% NaCl solution was added. The data have been obtained using the synchrotron radiation of LURE on station D16 (Paris-Sud University). A single bent germanium crystal allows work with a low background beam ($\lambda = 0.161$ nm). The photographs have been obtained using a STOE Explorer Camera in fixed geometry (crystal-film distance 75 mm) with exposure times as long as 30 min whereas the recording of the Bragg reflections needed only a few seconds; the diameter of the collimator was 0.5 mm. About 30 still patterns, at a resolution of 0.22 nm, have been obtained for various crystal orientations, precession angle values and positions during the 'precession motion'. This set of patterns, which includes all the major zones, is normally sufficient to exclude the possibility of having missed any major diffuse intensity. Four intensity components can be observed. The blackest spots are Bragg reflections. Each Bragg spot is surrounded by a diffuse scattering area which is the thermal diffuse scattering (TDS). When the Ewald sphere intersects a TDS area without intersecting the corresponding Bragg reflection a diffuse spot is recorded. The third component is the large diffuse ring (maximum around 3.0 nm^{-1}) which is due to the sample holder (capillary glass) and to the liquid water within and surrounding the crystal. The fourth component is the diffuse scattering which gives evidence of a disorder specific to the compound; it is mainly visible within the resolution range 0.8 nm-0.25 nm. For this figure, the 'vertical' rows are the traces of reciprocal planes perpendicular to c* and the 'horizontal' ones of planes perpendicular to a*; diffuse scattering is visible along both types of rows. The intensity asymmetry between the left and right sides of the film is due to the fact that this film was not perpendicular to the incident beam (it has been recorded with the precession angle 20°). The corresponding intensity corrections have been taken into account in our calculations.

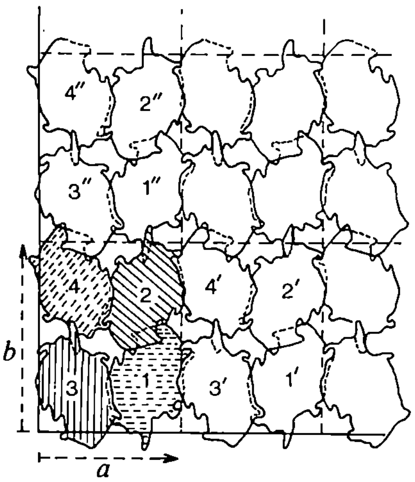

Fig. 2 Projection of the molecular envelopes onto the (**a**, **b**) plane. The unit cell contains four molecules: 1, 2, 3 and 4. The structure appears as alternate rows of molecules type (3-1-3′-1′...) and (4-2-4′-2′...) parallel to **a**, and (1-2-1″-2″...) and (3-4-3″-4″...) parallel to **b**. Only those parallel to **a** are real ones as their molecules have the same height along **c**, whereas the molecules forming the rows parallel to **b** have not. Along the **c** direction the structure can be also viewed as the juxtaposition of four types of rows (molecules 1-1..., 2-2..., 3-3..., 4-4...).

|**a**| for the **a** rows and (7±1) |**c**| for the **c** rows. We shall call 'unit' the set of atoms belonging to one period along a row (it may be part of molecule, molecule or group of molecules) and 'superunit' the whole atomic content corresponding to one row, that is, four units *a* aligned in the **a** direction (superunits *a*) or seven units *c* aligned in the **c** direction (superunits *c*). The atomic content forming the units and the corresponding displacements have been determined by comparison between film simulations and experimental diffuse intensities. One example of simulation is given in Fig. 3. Three terms have been included in the simulations: the Bragg intensity I_{B}, and two I_{D} terms, $I_{\mathrm{D(TDS)}}$ and $I_{\mathrm{D(D)}}$. $I_{\mathrm{D(TDS)}}$, which is located around each Bragg reflection, is due to the propagation of collective modes in crystals (thermal diffuse scattering, TDS).

For independent displacements:

$$I_{\mathrm{D(D)}} = \sum_n K_n(\langle|F_n|^2\rangle - |\langle F_n\rangle|^2) \qquad (1)$$

where $\sum_n$ sums over the various types of superunits, K_n is a factor which takes into account the total number of individual superunits and F_n is the scattering factor of the *n*th superunit, which may be written

$$F_n(\mathbf{S}) = \sum_p \left[\sum_{j=1}^{J_n} f_j(\mathbf{S}) \exp(-2i\pi\mathbf{S}\cdot(\mathbf{r}^j + \mathbf{u}_p^j + \mathbf{u}_n^j)) \times \exp(-2i\pi\mathbf{S}\cdot p\mathbf{d}_n) \right] \qquad (2)$$

where $\sum_p$ sums over the units of superunit *n* which are characterized by the correlation periodicity $\mathbf{d}_n$ (**a** or **c**), and $\sum_j$ sums over the J_n atoms of the unit associated with superunit *n*; $f_j(\mathbf{S})$ is the atomic scattering factor of atom *j*. The displacements $\mathbf{u}_p^j$ give rise to TDS and only the $\mathbf{u}_n^j$ which are correlated within a superunit are of interest. Satisfactory simulations have been obtained with small amplitude and harmonic rigid-body displacements, that is, with translations $\mathbf{u}_n$ of the whole superunit. In that case, (1) and (2) lead to the formula:

$$I_{\mathrm{D(D)}} = K_n|\langle F_n\rangle|^2(1 - \exp(-4\pi^2\langle(\mathbf{S}\cdot\mathbf{u}_n)^2\rangle)) \qquad (3)$$

Our results can be summarized as follows. The displacement directions are parallel to **a** for superunits *a* and to **c** for superunits *c*, with a precision of ±15°. With regard to the superunit contents, the best agreement is reached assuming two types of superunits *a*, one for which the unit contains molecules 1 and 3 and the other molecules 2 and 4 (see Fig. 2). There are also two types of superunits *c*, one for which the unit contains molecules 1 and 2, and the other molecules 4 and 3″. The last parameter extracted from our films is the amplitude of the displacements, from the comparison of the Bragg spots and the diffuse scattering intensities. This can be calculated from (3) by introducing the resolution effects and the total number of each type of superunit. With these conditions we get $\langle \mathbf{u}_a^2\rangle = (5\pm3)\ 10^{-4}\,\mathrm{nm}^2$ for the *a*-superunits, and $\langle \mathbf{u}_c^2\rangle = (7\pm3)\ 10^{-4}\,\mathrm{nm}^2$ for the *c*-superunits.

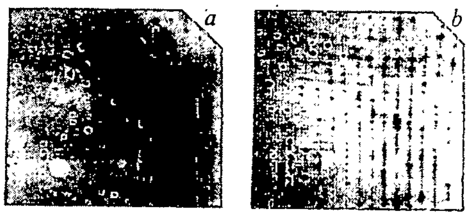

Fig. 3 Example of a comparison between experimental film (*a*) and simulation (*b*). The film intensity is simulated with the pixel density: 2,333 pixels nm^2, that is by 50,000 pixels. For each pixel, the intensity is the sum of three components: I_{B}, $I_{\mathrm{D(TDS)}}$ and $I_{\mathrm{D(D)}}$. I_{B} is zero except on the Bragg spots. $I_{\mathrm{D(TDS)}}$ is calculated from the approximation of isotropic elastic constants: $I_{\mathrm{D(TDS)}} = C|\langle F\rangle|^2\,\mathbf{S}^2/q^2$, where *q* is the distance of the extremity of **S** to the closest reciprocal node, $\langle F\rangle$ is the mean structure factor and *C* is a factor depending on the elastic constants. The experimental resolution is taken into account for I_{B} and $I_{\mathrm{D(TDS)}}$. The component $I_{\mathrm{D(D)}}$ is calculated according to equations (2) and (3). $I_{\mathrm{D(TDS)}}$ and $I_{\mathrm{D(D)}}$ are empirically scaled to I_{B} (the quantitative measurements of $I_{\mathrm{D(D)}}$ with respect to I_{B}, to determine the $\langle u^2\rangle$ values, have required additional patterns obtained with various exposure times). Finally the total intensity is corrected for the polarization effect according to ref. 34 and for the asymmetry effect due to the precession angle. The graphic drawing is done using sixteen grey tones. The parameters which have been varied are mainly the nature of the molecules forming the unit content, the direction of the displacements and the superunit lengths. Our simulations are quite sensitive to all these parameters; nevertheless, in some cases, the distinction between various unit contents has required a careful comparison between the experimental films and the simulations. In this orientation, **a*** lies in a vertical plane and **c*** in a horizontal plane. The Bragg spot and TDS intensities are not in complete agreement because of a small disorientation between the experiment and the film. The intensity modulations along the diffuse planes are in agreement except in the centre of the simulation (up to 0.8-nm resolution) which is overestimated. This effect is due to the solvent within the crystal (about 33% in mass) which has not been taken into account in our calculations.

Our results prove the existence of intermolecular rigid-body displacements which are correlated within short periodic rows of aligned molecules along **a** and **c**. A satisfactory aspect of the results is that the superunits have a physical meaning, namely that the molecules forming a superunit are sterically coupled. For instance, Fig. 4 shows that the coupling between molecules 1 and 3 in the **a**-direction is ensured by many contacts; in contrast, no contact between molecules 3 and 4 exists, which explains the absence of correlation in the **b** direction. Regarding superunits *c*, the coupling between molecules 1 and 2 (or 4 and 3″) is also plausible, as there is an overlap of these molecules looking along **c** which implies coupled displacements. It is difficult to determine the origin of the correlated displacements. It seems unlikely that they arise from a hinge-bending mode about an axis located in the (**a**, **c**) plane[23] as the displacements would display a component in the **b** direction, which is not observed. A possible cause is a local disorder, involving one or more residues in contact with a neighbouring molecule, which would produce a displacement of the latter, all displacements being correlated within a superunit. Such phenomena occur perhaps at the intermolecular contact between residues Asp 101

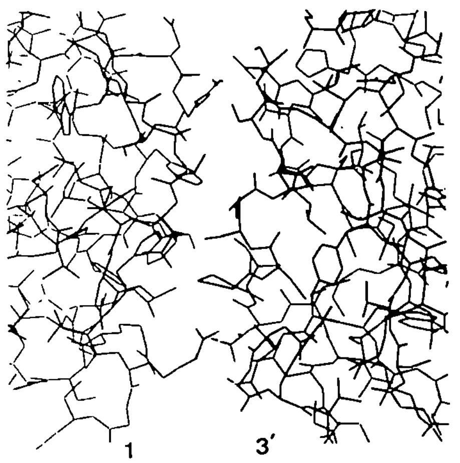

Fig. 4 Contacts between molecules 1 and 3′. The two molecular envelopes are complementary over an area of ~0.28 nm^2. About 12 intermolecular contacts <0.4 nm (involving residues Asn 113, Arg 114, Asp 119, Arg 125 of molecule 1 and Asn 65′, Asp 66′, Gly 67′, Ser 81′, Thr 89′ of molecule 3′) ensure a coupling between the molecules. The 15-20 well-localized water molecules in the intermolecular zone reinforce this coupling.

and Asn 103 of molecule 1 and Thr 47 and Asp 48 of molecule 2, which is characterized by distances between the mean atomic positions which are smaller than the van der Waals' distances.

We have shown that X-ray diffuse scattering can provide information not accessible by classical crystallography. In the case of hen egg-white lysozyme in the orthorhombic form, correlated rigid-body displacements of several molecules have been detected. It must be emphasized that, for this study, the diffuse scattering provides direct information only about rigid-body displacements of the molecules, that is intermolecular motions and intermolecular contacts. It is very likely that other protein crystals display diffuse scattering features due to intramolecular motions (such as domain motion). Their analysis can be performed in a similar way as the one reported here; our study must be considered as an initial study to develop a new approach to ptotein dynamics.

We thank J. Berthou and A. Lifchitz for lysozyme crystals, for access to their lysozyme data and for helpful comments. We also thank G. Bricogne and R. Fourme for stimulating discussions, S. Megtert for collaboration during the experiments, J. Cherfils for her help with graphic display work, V. Luzzati for reading the manuscript and C. Sommers for revising the English Manuscript.

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Erratum

The *Tetrahymena* ribozyme acts like an RNA restriction endonuclease

Arthur J. Zaug, Michael D. Been & Thomas R. Cech

Nature **324**, 429-433 (1986).

In this article corrections for the last four paragraphs were not incorporated on page. The corrected version is printed here.

Sequence-specific endoribonucleases might have many of the same applications for the study of RNA, that DNA restriction endonucleases have for the study of DNA[6]. The pattern of restriction fragments could be used to establish sequence relationships between two related RNAs, and large RNAs could be cleaved to fragments of a size more useful for study. The 4-nucleotide specificity of the ribozyme is ideal for cleavage of RNAs of unknown sequence; an RNA of random sequence would have an average of 1 cleavage site every 256 bases. The automatic end-labelling of one fragment during ribozyme cleavage is a practical advantage.

Development of the ribozyme as a useful tool for molecular biology has just begun. The cleavage efficiency of large RNA substrates needs to be improved, so that digestion is complete. The effects of denaturants such as urea and formamide must be further explored; they appear to increase the specificity of cleavage, and at the same time they should melt the secondary structure of the substrate to maximize the availability of target sequences. Finally, mutagenesis of the active site of the ribozyme must be continued to ascertain how many of the 256 possible tetranucleotide cleavage enzymes (of which we have investigated only four) work with acceptable efficiency and specificity.

Some protein ribonucleases cleave RNA substrates with high specificity by recognizing a combination of RNA structure and sequence; the structure is more important (for instance RNase III (ref. 28) and RNase M5 (ref. 29). On the other hand known proteins that cleave single-stranded RNA substrates are only specific for mononucleotides or dinucleotides (for instance, RNase T_1 cleaves after guanosines[30]). Thus, the L-19 IVS RNA is considerably more sequence-specific for single-stranded RNA cleavage than any known protein ribonuclease.

RNA sequence recognition often involves recognition of RNA by RNA. Triplet codons in messenger RNA are recognized by anticodons in transfer RNA, and ribosome binding sites in prokaryotic mRNA are recognized using the Shine-Dalgarno interaction with 16S rRNA. It is easy to imagine uses for sequence-specific protein ribonucleases in pre-mRNA splicing and 3′-end generation, but there is no evidence for such enzymes. Instead, 5′ splice sites in pre-mRNA are recognized using the U1 small nuclear RNA[13,31] and the 3′ end cleavage site of histone H3 pre-mRNA by U7 snRNA[32]; much of the sequence discrimination is provided by complementary base-pairing. Perhaps it is difficult to construct an active site in a polypeptide to bind an unstructured stretch of nucleotides specifically. It may be possible that in certain reactions involving RNA substrates, RNA catalysis has not been superseded by protein catalysis for the simple reason that RNA does the job better.

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PERTH WA

CSIRO conducts scientific and technological research in laboratories located throughout Australia and employs about 7400 staff, of whom some 2,800 are professional scientists. As Australia's main strategic research organization, CSIRO's stated role is research in support of major sectors of the Australian economy, and the effective transfer of its results to users.

GENERAL: Dryland Crops and Soils is a multidisciplinary research program established in 1984 by the Divisions of Plant Industry and Soils. Based in the Laboratory for Rural Research in Perth, Western Australia, the program aims to provide understanding of and management options for improved crop production in coarse textured and texture-contrast soils in Mediterranean climate zones of Australia. In areas of the wheat-growing regions of WA, root diseases severely limit crop production. Take-all (*Gauemannomyces graminis*) is one of the major root diseases limiting the production of cereals, particularly wheat.

DUTIES:In collaboration with the crop physiologists, root biologists and soil scientists in the program, the appointee will be expected to initiate research in the field and glasshouse aimed at understanding the influence of soil management and environmental factors (mineral nutrition and water status), on the physiological basis of susceptibility of wheat to take-all (*Gauemannomyces graminis*).

QUALIFICATIONS:A PhD degree or equivalent qualifications in plant pathology. Experience with root pathology and root biology would be preferred. A good background in plant physiology/biochemistry is essential.

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting reference No A4632, should be directed to:

The Chief
CSIRO Laboratory for Rural Research
Private Bag
PO Wembley WA 6014
by March 20, 1987.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W3806)A

FACULTY OF PHARMACY
UNIVERSITY OF TORONTO

Assistant or Associate Professor

Applications are invited for a tenure-stream post beginning September 1, 1987. The successful candidate will be expected to teach Medicinal Chemistry at the undergraduate and graduate levels and to develop an independent research program in an area related to the structural and mechanistic determinants of drug action. Individuals with interests and experience in fields such as genetic engineering, computational molecular biology, protein chemistry, or receptor biology are particularly encouraged to apply. Candidates must hold a Ph.D. degree or its equivalent and have at least two years of postdoctoral experience.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and landed immigrants.

A curriculum vitae, a one-page outline of a future research program, and three letters of reference should be forwarded by May 15, 1987 to:

D.G. Perrier, Dean
Faculty of Pharmacy
University of Toronto
19 Russell Street
Toronto, Ontario
Canada M5S 1A1

(NW495)A

INTERNATIONAL JUTE ORGANISATION

SENIOR RESEARCH ADVISER

General: Applications are invited for the position of a Senior Research adviser to assume responsibility for the work of two Sub-projects "Collection, Conservation, Characterisation and Exchange of Germplasm for Development of Improved Varieties of Jute, Kenaf and Other Allied Fibres" under the general direction of Executive Director and Director Projects and in cooperation with Senior Agriculture Officer of IJO. The canadidate will be responsible for administration, supervision, management and implementation of the Sub-projects including preparation of detailed work plan; providing assistance in establishing the new work programme; advising national institutions of participating countries and fielding the exploration teams for germplasm collection; organising training and workshops and advising E. D. to ensure smooth implementation of the Sub-projects and effective utilisation of the services of specialised consultants and other programme inputs funded from project resources; field inspection and on the spot investigation of the problems; evaluation of the factors leading to the shortcomings and assisting IJO in planning and implementing the steps necessary to overcome the problems; establishment of direct contact with officials and authorities concerned with preparation and implementation of the projects in the field; preparation of periodic reports, annual reports, technical reports, and terminal report.

Qualifications: University degree in agriculture science preferably a Ph.D. degree; 10 years of professional experience in variety development in addition to planning and executing agriculture projects in Governmental Agencies or International Organisations; supervision of staff and at least 5 years of practical experience in (1) germplasm collection, characterisation and conservation and (2) in the breeding of crop plants. The applicant should have the knowledge of programmes and procedures for implementation of agricultural projects under the conditions and problems of developing countries; the ability to plan, organise, coordinate and evaluate the work; willingness and fitness to travel and undertake field inspections, particularly in the jute and kenaf growing areas of Asia.

Language: Good knowledge of English; knowledge of other languages desirable.

Salary: The basic salary (P4/P5) will be in accordance with the applicant's qualifications and experience. Terms and other benefits as applying to UN organizations will also be admissible. The position will be based at IJO, Dhaka, Bangladesh.

Tenure: The post is available for a fixed term of two years.

Two copies of the Applications along with 2 recent photographs of the candidates should be directed to: The Executive Director, International Jute Organisation (IJO), 95A, Road 4, Banani P.O. Box No. 6037, Gulshan, Dhaka, Bangladesh.

Closing date for receiving applications is: 15 April 1987. (W3808)A

Biosensor Breakthrough

Molecular Devices Corporation is developing novel silicon-based, chemical sensor technology. This new sensor can quantitate receptor activity in reconstituted biological membranes and thus provide an electrical response to biological receptor functions. This represents a major scientific advancement and would permit rapid screening and analysis of pharmacological agents such as receptor agonists, antagonists, and toxins.

Membrane Biophysicist

We are looking for a PhD scientist with research expertise in membrane biophysics or membrance biochemistry to play a major role in this interdisciplinary research. General knowledge of analytical chemistry and electrochemistry is desired.

To apply, please send your résumé to Human Resources, Molecular Devices Corporation, Dept. N-1, 3180 Porter Drive, Palo Alto, CA 94304. An equal opportunity employer. (NW490)A

Molecular Devices

POSTDOCTORAL RESEARCH ASSOCIATES, PHYSICAL CHEMISTRY OR CHEMICAL PHYSICS.

Two positions available immediately for recent Ph.D.'s in physical chemistry or chemical physics to work in areas of gas-phase molecular dynamics, spectroscopy and photoionization. Some areas of current research include: (1) high resolution infrared spectroscopy of chemical intermediates produced by pulsed discharge or UV laser photolysis; (2) photoionization dynamics of resonantly excited molecules by photoelectron and ion mass spectroscopies; (3) state-resolved reaction dynamics using laser or synchrotron radiation probes. Extensive experimental facilities are available including excimer and Nd:YAG pumped dye-laser systems, photoelectron and ion spectrometers; and VUV beam lines located at the National Synchrotron Light Source (NSLS).

Candidates should send résumé and three letters of recommendation to: **Dr. Alfred P. Wolf, Chairman, Chemistry Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, L.I., New York 11973. Equal Opportunity Employer.** (NW496)A

UCG Coláiste na hOllscoile Gaillimh

UNIVERSITY COLLEGE GALWAY

IRELAND

JUNIOR LECTURESHIP IN MATHEMATICS

Applications are invited for the above full-time post. Salary Scale: IR£11,255 × (9) IR£15,503.

Closing date for receipt of applications, 19 March, 1987. Further information may be obtained from the Registrar, University College, Galway, Ireland. (2080)A

POSTDOCTORAL POSITION

available in muscle physiology/exercise physiology/animal locomotion. To participate in a multidisciplinary project designed to understand how muscle functions during locomotion in fish, frogs, and mammals. Involves single muscle fiber mechanics, histochemistry, electrophoresis, EMGs, oxygen consumption, and functional morphology (This position may involve work in Leeds, England with R. McNeill Alexander, or work in the United States, or both). Send curriculum vitae to: **Dr Larry Rome, Department of Zoology, University of Tennessee, Knoxville, TN37996.** *University of Tennessee is an Equal Opportunity Employer.* (NW483)A

GENETICIST UNIVERSITY OF ILLINOIS

In reference to the above position, advertised in *Nature* January 15 and 22, 1987: To receive full consideration, applications should be received by March 15, 1987.

(NW482)A

POSTDOCTORAL POSITION

Available immediately to investigate the mechanism o action of insulin and the hormonal control of protein phosphorylation in skeleta muscle and fat cells. Salar $17,000—$22,000 per yea depending on experience Send resume to: **Dr John C Lawrence, Department o Pharmacology, Washington University School of Medicine 660 South Euclid, St. Louis MO 63110.** (NW474)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following positions:

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
Department of Medicine and Clinical Science

POSTDOCTORAL FELLOW/RESEARCH FELLOW

(Molecular Biologist/Protein Chemist)

The Department seeks to appoint a Postdoctoral Fellow or Research Fellow with a background in Molecular biology and/or Protein Chemistry, to work with the mucosal group which has established interests in the cell biology of inflammation, immunity and cancer relevant to human intestinal disease. Current projects include the regulation of plasminogen activator by transforming human colon epithelial cells, growth factor production by lamina propria cells and antigen presentation in the intestine by dendritic-like cells. Close collaboration exists with basic research groups in the School, including cancer biology, developmental haematology and medical molecular biology.

Closing date: 20 March 1987 Ref: JC.21.1.1.

RESEARCH SCHOOL OF PACIFIC STUDIES
Department of Biogeography and Geomorphology

GEOMORPHOLOGIST

POSTDOCTORAL FELLOW/RESEARCH FELLOW OR SENIOR RESEARCH FELLOW

Applicants should be geomorphologists with research experience in fluvial processes and landforms. Numerical modelling abilities and interests in semi-arid environments are desirable but not essential. The post is available from 1 August 1987. Interested persons must seek further information from Professor D. Walker (062) 492235 or Dr J.M.A. Chappell (062) 494340.

Closing date: 27 March 1987 Ref: PA.21.1.1.

CONDITIONS OF APPOINTMENT: Salary will be in accordance with qualifications and experience within the range: Senior Research Fellow \$A38,917—\$46,420 p.a.; Research Fellow \$A27,859—\$36,600 p.a.; Postdoctoral Fellow Grade 1 (at a fixed point) \$A24,013—\$27,507 p.a. Appointment, unless otherwise stated, will be: Senior Research Fellow/Research Fellow normally up to three years with the possibility of extension to maximum of five years; Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years. Grants are provided towards travel and removal though special conditions may apply to persons appointed to externally funded positions. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Applicants should quote the advertisement reference when requesting further particulars and when submitting applications, which should be forwarded to the Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia. Further particulars also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W3812)A

FACULTY POSITION/STABLE ISOTOPES/ UNIVERSITY OF CALIFORNIA, SANTA BARBARA

The Department of Geological Sciences, University of California Santa Barbara is seeking candidates for a tenure track faculty position in the area of light stable isotopes especially in marine related science. The successful candidate will be responsible for teaching and research in the applications of light stable isotopes in such areas as marine and terrestrial geology, marine science general; and in graduate and undergraduate teaching. The university will soon develop a light stable isotope laboratory. Candidates must possess a Ph.D. in geology or oceanography with a demomstrated record of research accomplishments. Rank and salary are negotiable. The position is available effective July 1, 1987.

Applicants should send résumé and name of five referees to **Dr. Michael Fuller, Chairperson, Department of Geological Sciences, University of California, Santa Barbara, CA 93106.** Applications will be reviewed beginning March 20, 1987 and thereafter unitil the position is filled. *The University of California is an Equal Opportunity/ Affirmative Action Employer.* (NW489)A

Moving forward in life sciences

Research scientist

Kodak's market pre-eminence across a range of photographic products is the hallmark of our commitment to innovative, forward-looking research and development. A commitment which is fully reflected in our increased activity in the development of new materials and technologies for the biological and biomedical industries.

We now urgently require an ***immunologist/cell biologist*** to join a new and expanding multi-disciplinary team investigating the interaction of cells and biological macromolecules with surfaces. A BSc in immunology with at least five years' relevant experience or a PhD with two years' experience are the minimum requirements for this important position. To join this group you must have a good background in cell culture and immunological techniques, while some industrial experience would be an advantage.

In addition to conducting high quality research, the scope that will be provided for converting ideas into marketable products makes this an ideal opportunity to gain exciting commercial experience within a competitive environment.

To develop successfully in this permanent position, you will need to be both creative and self-motivating and able to make a significant contribution within your specialisation.

We can offer a salary of £11,000 - £15,000 to a person of the right calibre and age together with the kind of generous benefits package you would associate with a multi-national organisation.

For further information please send a full c.v. to Tony Frankland, Kodak Limited, Headstone Drive, Harrow, Middlesex HA1 4TY.

(2097)A

Kodak

1986

Yale University School of Medicine Section of Neuroanatomy

ASSISTANT PROFESSOR

An Assistant Professor position is available for a neurobiologist with experience and interest in electrophysiology and cytochemistry of neurons using *in vitro* slice preparation. Teaching responsibilities include medical and graduate courses. Candidate is expected to form an independent laboratory. Send resume to: Chairman, Section of Neuroanatomy, Yale University, School of Medicine, 333 Cedar Street, New Haven, Ct. 06510.

An Equal Opportunity/Affirmative Action Employer

(NW470)A

Laboratory Technician
Falkland Islands

The Falkland Islands Agricultural Research Centre is intending to increase the productivity of Falkland Island sheep farming based on wool production. A multi-discipline team of Agronomists, Sheep Husbandry Specialists, a Veterinary Investigation Officer and an Ecologist provide the main input.

The successful Laboratory Technician will work in a laboratory involved in veterinary diagnostic work as well as nutrition chemistry. Responsibilities include analysis of herbages, soils, fertilisers, organic manures and feeding stuffs; maintaining live animals for providing rumen liquor for in vitro digestability determinations; and assisting in other laboratory work.

Applicants should be British Citizens who are qualified Laboratory Technicians with HNC/D in chemistry or similar and have 5 years experience in proximate analysis of herbage samples. Familiarity with analysing soils, fertilisers, organic manures and feeding stuffs is essential.

The appointment is on contract to ODA, on loan to the Government of the Falkland Islands, for a period of two years. Salary (UK taxable) will be commensurate with qualifications and experience and will include an element in lieu of superannuation. A tax free Foreign Service Allowance, currently in the range £235 to £2,325 pa, is also payable.

The post is wholly financed by the British Government under Britain's programme of Aid to the developing countries. Other benefits normally include paid leave, free family passages, children's education allowances, free accommodation and medical attention.

For an application form, please write, quoting ref. AH369/DF/N, **stating post concerned,** to: Appointments Officer, Overseas Development Administration, Room 351, Abercrombie House, Eaglesham Road, EAST KILBRIDE, Glasgow G75 8EA. Or telephone 03552 41199, extension 3533. (2058)A

ODA OVERSEAS DEVELOPMENT

Britain helping nations to help themselves

MEDICAL RESEARCH COUNCIL

An Equal Opportunity Employer

MRC CLINICAL & POPULATION CYTOGENETICS UNIT

NON CLINICAL SCIENTISTS FOR PARALLEL COMPUTING

Two short term appointments are available on the staff of the MRC Cytogenetics Unit's Pattern Recognition & Automation Section, on Edinburgh. The positions, one for two and one for three years, are open to postdoctoral (or equivalently qualified) candidates with a strong interest in parallel processing of digital images. The appointments offer an excellent opportunity to develop both theoretical and practical solutions to real problems in various fields of medical imaging from light microscopy of cells and chromosomes to CT and NMR scanning. The parallel processor environments that are available comprise a 96×96 parallel array processor, and a number of loosely coupled multiple Motorola systems. Processing close to the image is done in procedural languages (C, and where necessary on the array processor, assembler); higher level control will be by an intelligent knowledged based system. The research envisaged will be part of a team effort in the development of intelligent microscopy on the one hand, and of an Alvey Consortium working on advanced methods of analysis of medical images on the other.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Applicants should send a curriculum vitae and letter of application by 20th February, 1987 to Mr A.H. Douglas, Administrative Officer, MRC Clinical & Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, from whom further information may also be obtained. Please quote reference PR/2/87. (1978)A

MRC MECHANISMS IN TUMOUR IMMUNITY UNIT

MRC CENTRE

Hills Road, Cambridge
CB2 2QH

CAREER APPOINTMENT IN PROTEIN CHEMISTRY/ IMMUNOCHEMISTRY

Applications are invited for a protein/immunochemist to work in the above Unit in the first instance. Applicants should have experience, preferably in the complement field, of protein isolation, characterisation and assay and in the analysis of protein-protein interactions. Experience with FPLC and HPLC systems an knowledge of the techniques used in the analysis of membrane proteins, in protein sequencing and in monoclonal antibody production would also be advantageous though not essential.

In addition to maintaining an independent research programme within the general area of the regulation of expression of complement activity and complement mediated functions, the appointee will be expected to participate in the supervision of both predoctoral and postdoctoral research and to maintain a stock c complement proteins and reagent for use within the Unit.

The post, which is availabl from 1 October 1987, will be at a appropriate point on the MR non-clinical scientific staff salar scale (equivalent to the Universit Academic scale) according to age qualifications and experience.

Further details from and en quiries to Professor P.J. Lach mann (0223 243237), to whom applications, including a full c.v publications list, names of tw referees and a statement of n more than 500 words outlining th applicant's proposed researcl should also be made by 5th Marc 1987.

The Medical Research Counc is an Equal Opportunity E ployer. (2051)A

Tenured Appointment in MOLECULAR IMMUNOLOGY

The Department of Microbiology of the University of Pennsylvania School of Medicine is planning to make a tenured appointment of an established scientist in the area of molecular immunology with expertise in protein chemistry. This appointment will involve the development of a research program which will complement existing programs in immunobiology and immunogenetics. The selected candidate will also participate in the educational activities of the medical school and biomedical graduate program. Only applicants with a substantial publication record of significant contributions and a successful record of competitive funding will qualify for consideration. The projected starting date is January 1988. Interested individuals should submit a curriculum vitae, bibliography, three or more references and a statement of future research plans to: **Dr Fred Karush, Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6076.** (NW466)A

NMR IMAGING PHYSICIST

The Department of Radiology of The Johns Hopkins University School of Medicine is seeking candidates to fill a tenure-track faculty position in NMR imaging. This position will emphasize clinical and research applications of NMR imaging and spectroscopy. Title, salary, and responsibilities of the candidate will be commensurate with his or her qualifications and experience. Available instrumentation includes 1.5T, 4.7T, and 8.5T systems, and vigorous pursuit of research and instrumentation development will be encouraged. Excellent opportunities exist for collaboration with physicians, biomedical scientists, and spectroscopists. Interested individuals should send a description of their research interests, curriculum vitae, and three letters of recommendation to **Martin W. Donner, MD, Professor and Chairman, Department of Radiology and Radiological Science, The Johns Hopkins Hospital, Baltimore, Maryland 21205.** (NW475)A

UNIVERSITY OF OXFORD

Laboratory of Molecular Biophysics

ıpplications are invited for the ost of

RESEARCH ASSISTANT IN PROTEIN CRYSTALLOGRAPHY

› develop the use of a commercial vo-dimensional area detector for data collection from protein rystals and to participate in ructural studies utilising the right synchrotron radiation ›urce. The post is funded by n MRC Programme Grant and is vailable from 1 April, 1987 to 30 eptember, 1991. Salary is accord-ıg to age and experience on the :ale IA.

The Laboratory of Molecular iophysics is currently up-dating s X-ray diffraction apparatus ith the purchase of a commercial rea detector. The work will ıvolve improvements to existing ›ftware and to the diffraction pparatus in order to provide a ıst, precise and efficient data ›llection facility for protein ·ystals. It is anticipated that ıe research assistant will be ssociated with one of the on-›ing structural research projects ıd will be encouraged to carry ut original research.

Applicants should have a octorate or similar qualification ı protein crystallography or in :lated fields such as crystal-›graphy, computational physics r engineering. Proven ability in ›mputer programme is essential. pplications together with a curri-ılum vitae and the names of two :ferees should be sent to Prof ır David Phillips, Laboratory f Molecular Biophysics, Rex ichards Building, South Parks oad, Oxford OX1 3QU by 7 [arch, 1987. (2079)A

UNIVERSITY OF MANCHESTER

POSTDOCTORAL RESEARCH ASSOCIATE IN RHEUMATOLOGY

pplications are invited for this ›st, in a unit studying mechan-ms of angiogenesis. The unit is ıpported by the Wolfson Foun-ıtion and consists of biochemists ıd cell biologists. Experience in ther cell culture techniques, rowth factor research (particu-ly receptor studies) or connec-ve tissue biochemistry would be ·eferred but is not essential. ılary is in the range (under ·view) £8,020—£10,865 p.a. :pending on age and experience.

Appointment is for one year in e first instance, renewable for a rther two years. Please write to r J.B. Weiss, Rheumatic Dis-ıses Centre, University of [anchester Clinical Sciences uilding, Hope Hospital, Salford [6 8HD. (2077)A

UMIST

DEPARTMENT OF BIOCHEMISTRY AND APPLIED MOLECULAR BIOLOGY/ THE MANCHESTER BIOTECHNOLOGY CENTRE

Molecular Genetics and Biochemistry of *Streptomyces*

POSTDOCTORAL RESEARCH ASSOCIATES

(starting salary up to £9,495 p.a. under review)

2 POSTGRADUATE RESEARCH ASSISTANTS

(starting salary up to £8,505 p.a. under review)

These are three year posts (to start July 1987) funded jointly by the SERC, DTI and four industrial companies under the special initiative 'Antibiotics and Recombinant DNA Technology'. One post (postdoctoral) is to work on transposition in *Streptomyces*. The others are to work together with microbial physiologist on a study of the molecular mechanisms of the switch between primary and secondary metabolism. The successful candidates would ideally have some experience in the molecular genetics of *Streptomyces*, but if necessary appropriate training can be given. There is at present an active group of twelve people working on *Streptomyces* molecular genetics. The department is superbly equipped and possesses extensive experience in most aspects of molecular genetics.

Applicants should send a c.v. and names and addresses of two referees, quoting reference BIO/ R/12, to Dr John Cullum, Department of Biochemistry and Applied Molecular Biology, UMIST, P.O. Box 88, Manchester M60 1QD. Informal enquiries: (061) 236 3311 extn 2032. The closing date is 6 March, 1987. (2074)A

THE KENNEDY INSTITUTE

Applications are invited for a position as a postdoctoral Research Assistant in the Division of Biochemistry as part of a group investigating transfer of lysosomal enzymes between cells. The post is funded for three years by the Mental Health Foundation to investigate receptors for lysosomal enzymes on glial cells and transfer of cell surface glycoproteins between co-cultured cells. Experience in techniques of neural cell culture, enzyme assay and immunochemistry would be an advantage. Starting salary according to age and experience up to £12,162 including London Weighting.

Applications together with details of two referees should be sent to: The General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, 6 Bute Gardens, Hammersmith, London W6 7DW. (2035)A

Graduate Protein Scientists

Cheshire

Two graduate scientists are required to join the growing biotechnology activity in ICI's health care business areas. Successful applicants will join a multi-disciplinary group consisting of protein scientists, fermentation technologists, oligonucleotide chemists and molecular biologists engaged in the cloning, expression and isolation of genes and proteins of pharmacological interest.

Applicants should be aged between 22 and 35 with a degree or equivalent in chemistry, biochemistry or related discipline. The successful candidates will have experience in either protein isolation, purification and analysis in a research environment or in scale up to provide materials suitable for human administration.

We can offer excellent technical and recreational facilities situated in attractive Cheshire countryside about 15 miles south of Manchester. Our salaries and related benefits are highly competitive and generous assistance with relocation is available if appropriate.

Applications including a detailed c.v. should be sent to:-

The Personnel Officer (Ref GPS),
Imperial Chemical Industries PLC,
Pharmaceuticals Division, Mereside,
Alderley Park, Macclesfield,
Cheshire SK10 4TG.

ICI is an equal opportunities employer (2094)A

بسم الله الرحمن الرحيم

KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH

Kuwait Institute for Scientific Research (KSIR) is a nonprofit organization with a staff of more than one thousand employees engaged in applied research in the fields of environmental and earth sciences; food resources; engineering; petroleum, petrochemicals and materials and techno-economics.

KSIR has a vacancy for a

BIOENGINEER

Major duties: Conducts scale up and optimization studies for various industrial fermentation systems at pilot plant level. Develop process control and model for biological systems utilizing computerized fermenters. Initiates and develops contract research proposals related to scale up studies of biomass production, biosurfactant production and wastewater treatment systems.

Qualifications: Applicants should have a Ph.D. in Biochemical Engineering with minimum 5-7 years of experience in pilot scale continuous fermentation systems.

KSIR offers attractive tax free salaries commensurate with qualifications and experience and generous benefits which include gratuity, free furnished airconditioned accommodation, school tuition fees for children, contribution to a savings plan, six weeks annual paid vacation, airtickets, free medical care and life insurance.

Interested applicants are requested to send their Curriculum Vitae with supporting information not later than one month of this publication, to:

Mr Habeeb Al-Sahhaf, Personnel Manager, Kuwait Institute for Scientific Research, P.O. Box 24885 Safat, 13109 Safat, KUWAIT. (W3807)A

THE AUSTRALIAN NATIONAL UNIVERSITY
RESEARCH SCHOOL OF CHEMISTRY

Postdoctoral Research Positions

Applications are invited from suitably qualified men and women for appointment to postdoctoral research positions in the Research School of Chemistry. Current tenured Faculty and their major research interests are:

Professor A.L.J. Beckwith (Physical Organic Chemistry); Dr M.A. Bennett (Organo-transition Metal Chemistry); Dr R. Bramley (Photophysics and Magnetic Resonance); Dr L.R. Brown (Nuclear Magnetic Resonance); Dr N.E. Dixon (Protein Chemistry, Enzymology and Molecular Genetics); Dr D.J. Evans (Liquid State Chemical Physics); Dr G.A. Heath (Inorganic Electrochemistry); Professor B.G. Hyde, Dr A.C. McLaren (Inorganic Solid State Chemistry); Dr E.R. Krausz (Optical Spectroscopy); Dr J.K. MacLeod (Chemistry of Biologically-active Compounds, Mass Spectrometry); Professor L.N. Mander (Synthetic Organic Chemistry); Dr G.B. Robertson (Crystallography); Professor A.M. Sargeson (Coordination and Biomimetric Chemistry); Dr T.R. Welberry (Disordered Materials); Professor J.W. White (Solid State Molecular Science); Dr S.B. Wild (Synthetic Inorganic Chemistry).

Appointments will be made at the levels of Postdoctoral Fellow and Research Fellow. The level appropriate for each appointee will be determined by the University and will depend on qualifications and experience. Appointment as Postdoctoral Fellow will be for one or two years; appointment as Research Fellow will be for three years in the first instance. Salary will be within the ranges: Postdoctoral Fellow $A24,013—27,507 p.a.; Research Fellow $A27,859—36,600 p.a. Current exchange rates at $A1 : Stg 44p : $US 65c. Grants are provided towards travel and removal. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time. Applicants should quote reference number RC.14.1.1 when requesting further particulars and when submitting applications which should be forwarded to the Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia. There is no application form. Applicants should supply to the Registrar by *15 April 1987* a curriculum vitae, list of publications, and statement of research interests, together with two passport-size photographs, the names and addresses of three academic referees and the probable date on which the Postdoctoral Fellowship or Research Fellowship, if awarded, could be taken up. Further particulars on the research programmes, equipment and conditions of appointment may also be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W3809)A

DIRECTOR, ONCOLOGY CENTER

The Johns Hopkins University School of Medicine and **The Johns Hopkins Hospital** are seeking a physician-scientist to lead our Department of Oncology, which is also a Comprehensive Cancer Center. Applicants should have evidence of leadership qualities and demonstrated outstanding abilities in research and teaching as well as the capability to direct the service missions of the Center.

Please send letter of application, curriculum vitae and bibliography to: Dr. Richard J. Johns, Chairman, Oncology Search Committee, 720 Rutland Avenue, Baltimore, MD 21205.

Deadline date for submission: March 1, 1987

An Affirmative Action/Equal Opportunity Employer

(NW429)A

PLANT MOLECULAR BIOLOGY
ASSISTANT PROFESSOR

The Salk Institute for Biological Studies has an immediate opening for an Assistant Professor in the Plant Biology Laboratory. The successful candidate will be expected to develop a research program in Plant Molecular Biology/Molecular Genetics. Applicants should have a Ph.D. degree as well as postdoctoral experience.

We offer competitive salaries and a comprehensive benefits package. Qualified candidates should send curriculum vitae, an outline of research accomplishments and interests, and three letters of recommendation by March 31, 1987, to:

Chair, Plant Biology Search Committee
Salk Institute for Biological Studies
P.O. Box 85800
San Diego, CA 92138–9216
Equal Opportunity Employer m/f/h/v

(NW488)A

UNIVERSITY OF NEWCASTLE UPON TYNE

FACULTY OF MEDICINE

LECTURER IN EPIDEMIOLOGY (PAEDIATRIC ONCOLOGY)

Applications are invited for the established post of non-clinical Lecturer in the Department of Child Health. This new post has been financed by an endowment from the North of England Children's Cancer Research Fund. The successful applicant will be a member of the Academic Department of child health in the new Medical School, Framlington Place, Newcastle upon Tyne. The responsibilities will primarily be to join the paediatric oncology team and to carry out research into the epidemiology of childhood cancer.

Salary will be at an appropriate point on the Non-clinical Lecturer Scale: £8,020—£15,700 per annum, according to qualifications and experience.

No forms of application are issued but applications (three complete copies) giving full details of qualifications and experience together with the names and addresses of three persons to whom reference may be made, should be lodged with the Senior Assistant Registrar, (Establishments), University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, not later than 7th March 1987. Interested applicants are invited to discuss the post with Dr A. Craft, Director, Paediatric Oncology Unit, tel: 091 2325131. (2050)A

UNITED MEDICAL AND DENTAL SCHOOLS
GUY'S CAMPUS
LABORATORY SCIENTIFIC OFFICER

required to work in the Rheumatology Unit/Molecular Immunogenetics Laboratory on HLA class II antigen biochemistry with relevance to rheumatoid arthritis. Previous experience in cell culture is desirable together with a knowledge of immunochemistry and protein electrophoresis. This project is funded by the Arthritis and Rheumatism Council to September 1990. Salary in range £5,588—£8,986 plus £1,113, according to age, experience and qualifications.

For further details telephone Dr J.S.S. Lanchbury on 01 407 7600 extn 3509.

Please apply with full c.v. and names and addresses of two referees to Staffing Office, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH, quoting ref: G/MED/268.

Closing date: 27 February 1987.
(2052)A

UNIVERSITY OF LEICESTER
STARLINK APPLICATION PROGRAMMER

Applications are invited for a experienced programmer to d velop applications software in th area of X-ray Astronomy. Thi will be carried out, in Fortran, o the Starlink VAX 11/750 com puter in the Leicester Universit Physics Department. The wor will involve close collaborat with members of the Leicester ray Astronomy Group, with Sta link staff at the Rutherfor Appleton Laboratory and with th X-ray Astronomy Special Intere Group.

Applicants should be exper ence in the production of scientifi data analysis software and, thoug not essential, preferably have ha postgraduate experience in astr physics or in a related subject. U to 25% of the successful can didate's time may be spent o relevant, collaborative researc projects within the X-ray Astr nomy Group.

The post is tenable for thre years from the date of appoin ment and the salary will be at a appropriate point on the SER 1A scale (£8,020—£12,780, wit superannuation). A contributio towards relocation expenses ma be payable.

Applications with a c.v. and th names of two professional referee should be sent by 1st March 198 to Dr B.A. Cooke, X-ray Astr nomy Group, Physics Dept. Leicester University, Leicest LE1 7RH. (2061)A

INSTITUTE OF OBSTETRICS AND GYNAECOLOGY
ROYAL POSTGRADUATE MEDICAL SCHOOL
RESEARCH ASSISTANT

required for studies including (1 the development of techniques t biopsy human embryos for pre implantation diagnosis of geneti ally inherited diseases, and (2) th production of transgenic mice b the microinjection of embryo ster cells into blastocysts. Previou experience in micromanipulation mammalian embryology or tissu culture would be an advantage Salary negotiable depending o experience; post initially avail for two years but will be exten on external funding. The succes ful candidate will be expected t register for a higher degree.

Enquiries and application including c.v. and three refere ces, to Professor R.M.L. Winsto or Dr A.H. Handyside, Institu of Obstetrics and Gynaecolog Hammersmith Hospital, Du Ca Road, London W12 0HS by 28 February (01-743 2030 ext. 415 2385). (2057)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF PHYSIOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

There is a vacancy for a postdoctoral research fellow in the Department of Physiology, University of Dundee. The post is available for a period of three years. The project, funded by the Wellcome Trust, is an electrophysiological study of the normal perinatal development of ion and fluid transport across mammalian airway epithelia and will involve close collaboration with the Department of Child Health (Professor R.E. Olver) as part of a programme of research into the physiology of the pulmonary epithelium.

The applicant should hold a PhD degree. Training is available for candidates without previous experience in electrophysiology who may have expertise in other techniques appropriate to this project.

The salary will be on the Research Assistant 1A scale.

Further details may be obtained from:

Dr M.R. Ward, (Dept. of Physiology, Tel. Dundee 23181 Ext. 4583).

Applications with c.v. (2 copies) and the names of 2 referees to The Personnel Office, The University, Dundee, DD1 4HN. Please quote ref. EST/441/86/N. Closing date 12 March, 1987. (2063)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from candidates to join a well-equipped laboratory investigating the regulation to muscle contraction. Research is focused on the mechanism of myosin-linked regulation using a variety of methods, including rapid-reaction and magnetic resonance techniques. Current work on the characterization of long-lived complexes associated with the relaxed state (Nature 313, 696-697), will be extended to more organised systems. The role of the light chain subunits will be explored in a collaborative protein engineering programme.

Applicants should have a background in protein/physical biochemistry or muscle physiology. The post is tenable for up to 3 years on the RA1A scale (starting within the range £8,020—£9,495). Applications, including a curriculum vitae and the names of two referees, should be sent to C.R. Bagshaw, Dept. of Biochemistry, University of Leicester, Leicester LE1 7RH, from whom further details may be obtained.

(2036)A

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT

MOLECULAR BIOLOGIST

A post is available for a Molecular Biologist to work on projects that may include the molecular analysis of mutations in the mouse, the induction and analysis of transgenic mice and/or insertional mutagenesis. Preference will be given to established molecular biologists with an interest in mammalian genetics. The successful candidate should be able to work independently, though willing to collaborate closely with other scientists investigating the mutagenic effects of various agents, aspects of developmental biology, molecular analysis of the haemoglobin and other loci, and gene therapy. The work will be conducted in the Genetics Division which generates a wide variety of mutant mice.

This is a 5 year appointment with salary according to MRC non-clinical scientific scales, dependent upon experience.

The Unit is situated in a pleasant rural area convenient for Oxford, and the Genetics Division has good links with Oxford University. Hostel accommodation may be available.

Applications, together with full c.v. and names of two referees should be sent to the Personnel Officer, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD. Closing date March 9th.

The Council is an Equal Opportunity Employer. (2072)A

THE OPEN UNIVERSITY

FACULTY OF SCIENCE

RESEARCH FELLOW IN PLANT BIOCHEMISTRY/ MEMBRANE FUNCTION

Applications are invited for the post of research fellow in the Biology Department. The postholder will investigate the role of inorganic ions and ion currents in plant growth and development.

Applicants should hold or expect shortly to receive a PhD or equivalent qualification in biochemistry or biophysics and have experience in electrophysiology.

The post is available for three years. Salary will be within the 1A research fellow scale: £8,020—£12,780 p.a. (under review).

Applications forms and further particulars are available from Mrs B. Woodall, (Post 0127/2), Faculty of Science, Wimpey II, The Open University, Walton Hall, Milton Keynes, MK7 6AA, or telephone 0908-653993: there is a 24 hour answering service on 0908-653868.

Closing date for applications: 2nd March 1987. (2082)A

A Senior Position in Protein Science

Cheshire

We have an opening for an experienced protein scientist, at post-doctoral level, to take responsibility for the isolation and purification of recombinant proteins for clinical evaluation. The objective is to bring laboratory-scale processes to a scale appropriate to support clinical trials with the necessary attention to quality requirements. Previous experience in such a position will be a distinct advantage. The successful applicant will join a multi-disciplinary team covering nucleotide chemistry, protein science, molecular biology and microbiology.

We can offer excellent technical and recreational facilities situated in attractive Cheshire countryside about 15 miles south of Manchester. Our salaries and related benefits are highly competitive and generous assistance with relocation is available if appropriate.

Applications including a detailed c.v. should be sent to:-

ICI

The Personnel Officer (Ref SPS),
Imperial Chemical Industries PLC,
Pharmaceuticals Division,
Mereside, Alderley Park,
Macclesfield, Cheshire SK10 4TG.

ICI is an equal opportunities employer (2095)A

PEPTIDE CHEMIST

Biomeasure Incorporated, a company dedicated to the development of therapeutic peptides, is offering an excellent opportunity for a Peptide Chemist with the following credentials:

- PhD in Organic Chemistry with 3 to 5 years postdoctoral or industrial experience in peptide chemistry.
- An interest in structure activity studies, and a working knowledge of computer assisted drug design would be a plus.
- The proven ability to design, synthesize, purify and characterize biologically active peptides.
- An outstanding record of scientific accomplishments.

Located near Boston, Biomeasure Incorporated provides state-of-the-art facilities, and a supportive environment for the conduct of research including a close collaboration with an academic team renowned in the field of therapeutic peptides. Excellent compensation, benefits and relocation package available for qualified applicants.

Send resume and salary requirements in confidence to: **Human Resources, Biomeasure Inc., 11-15 Avenue E, Hopkinton MA 01748.**

(NW467)A

Biomeasure

an equal opportunity employer m f

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL
(UNIVERSITY OF LONDON)

Appointment of Dean

The Academic Board and Council of the Medical School propose to appoint a Dean on the resignation of the present Dean, Dr. R. J. West MB BS MD FRCP DCH DObst RCOG on 31 July 1987. The School will be flexible with regard to the terms and conditions of appointment (for example whether full-time or part-time) in order to place the highest priority on finding the most suitable candidate for the post which it is envisaged will be for a period of three years in the first instance renewable for a further period of three years. Persons, preferably medically qualified, of appropriate seniority with substantial experience of teaching, research and academic administration who might wish to be considered for the post, or persons wishing to bring such candidates to the attention of the Search Committee, are invited to write in confidence to Dr. J. A. Firth, Chairman of the Academic Board, Department of Anatomy, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, from whom further particulars are available. The Search Committee hopes to consider suitable persons by 31 March 1987. (2083)A

STAFF FELLOWS, MEDICAL STAFF FELLOWS, VISITING SCIENTISTS, and VISITING FELLOWS

Positions available at the Addiction Research Center (ARC) of the National Institute on Drug Abuse for recent, as well as experienced, doctoral level graduates in psychology, the neurosciences and pharmacology and for physicians trained in psychiatry or nuclear medicine. The 12 laboratories of the ARC are engaged in a wide range of research on the vulnerability to substance abuse, mechanisms of drug action, and the treatment of drug dependence. The ARC provides significant training opportunities in addition to the capacity to become involved in a body of individual and collaborative study. Research fellows are generally appointed for an initial two-year period which can be extended. Salaries depend on training and experience.

Applicants interested in exploring the opportunities presented by the ARC should write to:

Jerome H. Jaffe, M.D., Director, NIDA Addiction Research Center, 4940 Eastern Avenue, Baltimore, MD 21224 (NW492)A

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Industrial Biology, to

Felicity Lyons, Macmillan Journals, 4, Little Essex St.
London, WC2R 3LF

or

Paige Beaver, Bio/Technology, 65, Bleecker St.,
New York, NY10012, USA

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

Link Group with Scottish Crop Research Institute, Invergowrie

The AFRC has provided resources for three years to undertake a molecular analysis of potato leaf roll virus — a pathogen of considerable economic importance. A postdoctoral scientist has already been appointed and we now wish to recruit an appropriately qualified TECHNICIAN to participate in this project. The appointment is available from May 1987; Starting salary within range £5,968—£7,274 per annum on University Grade 3 or Grade 5 Technician scales according to qualifications and experience.

Candidates should have an HNC, advanced City and Guilds or equivalent qualification, together with appropriate experience.

Further information can be obtained by telephoning Dr R.T. Hay (0334 76161 ext. 296).

Applications (two copies preferably in typescript) with the names of two referees should be sent to the Establishments Officer, College Gate, University of St Andrews, Fife, KY16 9AJ to arrive not later than 19th February, 1987. (2060)A

SCOTTISH CROP RESEARCH INSTITUTE

Invergowrie, Dundee DD2 5DA

FORAGE BRASSICA BREEDING DEPARTMENT

HIGHER SCIENTIFIC OFFICER (BAND I)

3-year appointment

A vacancy exists within the Forage Brassica Breeding Department of the Institute for a Higher Scientific Officer who will be involved in the development of selection and screening methods using *in vitro* cell and tissue culture techniques and to apply these in an attempt to produce *Brassica napus* plants with novel herbicide resistance.

Salary scale: £8,405 in 1st year of appointment rising to £9,285 in 3rd year.

Qualifications: First or upper second class honours degree with at least two years post qualifying relevant research experience which may be a course of research and study leading to a PhD degree.

Non-contributory superannuation.

The Institute is an equal opportunities employer.

To apply please send curriculum vitae and the names of three referees to Miss J.A. Murray, Personnel Officer, by Monday 2nd March 1987. (2043)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF HUMAN ANATOMY AND CELL BIOLOGY

Two Posts of

POSTGRADUATE RESEARCH ASSISTANT/ POSTDOCTORAL SENIOR RESEARCH ASSISTANT

for Studies on the Dynami
Behaviour of Actin Filaments

Applications are invited for tw posts of Wellcome-funded an MRC-funded Research Assistan Senior Research Assistant to wor with a group studying the d namics and regulation of actin fil ment assembly in cells and *in vitr* The appointees will either follo the localisation of newly-polyme ized microinjected hapter labelled actin into single livin cells (MRC) or study the dynami polymerisation behaviour of acti filaments and their regulation b other cytoskeletal proteins (Well come). Both posts are tenable fo a period of three years from negotiable starting date. Inform enquiries may be made to Dr Sheterline, Tel. 051-709 6022 Ext 2842.

Initial salaries within the rang £7,055—£8,505 per annum (unde review). Applications, togethe with the names of three referees should be received not later tha 27 February 1987, by The Regis trar, The University, P.O. Bo 147, Liverpool, L69 3BX, fro whom further particulars may b obtained. Quote ref. RV/350.N. (2040)A

UNIVERSITY OF OTAGO

Dunedin, New Zealand

CHAIR OF MICROBIOLOGY

The University Council invite applications for appointment t the Chair of Microbiology. Th Department of Microbiology i administratively part of th Faculty of Medicine, but is als responsible for a full range o courses in the Faculty of Science Applicants with either medica or science qualifications will b considered.

Professorial salaries, which ar regularly reviewed are fixed a various points within the preser ranges: medically qualified sta NZ$80,000 to NZ$87,000, othe staff: NZ$62,000 to NZ$77,500.

Intending applicants are invite to write for further particulars available from the Secretar General, Association of Com monwealth Universities, (Appts) 36 Gordon Square, Londo WC1H 0PF; or from the Registra of the University, PO Box 56 Dunedin, New Zealand.

Applications close on 15 Apr 1987. (W3815)A

UNIVERSITY COLLEGE LONDON
FACULTY OF CLINICAL SCIENCES
DEPARTMENT OF MEDICINE

POSTDOCTORAL POSITION IN MOLECULAR BIOLOGY

Applications are invited from postdoctoral scientists to join an active group working on the molecular basis of Chronic Granulomatous Disease (CGD) and the biochemistry of the microbicidal oxidase electron transport chain of phagocytes. Considerable scope for independent research and advancement. Experience in molecular biology or biochemistry essential.

The post is available from 1 March 1987 or as soon as possible thereafter for three years in the first instance and is supported by the Wellcome Trust. Salary will be on Range 1A £9,880—£10865 + £1,393 LW.

Applications with c.v. and names of two referees should be sent to Prof. A.W. Segal, Department of Medicine, University College London, Rayne Institute, University Street, London WC1E 6JJ, from whom further information may be obtained by telephoning 01-380 9725. (2066)A

UNIVERSITY OF CAMBRIDGE
School of Clinical Medicine

RESEARCH SCIENTIST
Department of Surgery
Orthopaedic Research Unit

Required to research into the corrosion products from total joint prostheses and to assess their role into the causes of clinical reactions after joint replacement. The post involves clinical, biochemical, immunological and histological analyses of biological specimens obtained from patients and other models carried out in our laboratories to monitor the biocompatibility screening of new implant materials and the effects of bacteria, infection and the loosening of the joints. This post is to head a team under Mr Neil Rushton, Director of the Orthopaedic Research Unit.

This graduate post is supported on a grant from the DHSS for one year in the first instance. Salary range £9,880 - £15,700 according to age, qualifications and experience.

Applications, in writing, giving the names of two referees to: Mr R C Smith, Principal Assistant, Department of Medicine, Level 5, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. (2033)A

UNITED MEDICAL AND DENTAL SCHOOLS
POSTDOCTORAL RESEARCH ASSISTANT

Postdoctoral research assistant required for 3 years to work on a Cancer Research Campaign grant to investigate antibody and T-cell responses to human papillomaviruses. Applicants should have experience of the immunological techniques of ELISA and Lymphocyte transformation. The successful applicant would gain experience of viral DNA cloning and other molecular techniques, including use of bacterial expression vectors to produce viral proteins. He/she would be based in the Department of Virology at St Thomas's Hospital, but would spend some time in the Department of Microbiology, Guy's Hospital Medical School.

Further information from Dr J.M. Best, Department of Virology, St Thomas's Hospital, London SE1 7EH, Tel: 01-928-9292, ext 2453, or Dr D. McCance, Department of Microbiology, Guy's Hospital London SE1 9RT, Tel: 01-407-7600, ext. 3649.

Applications with full curriculum vitae and names of two referees, to the Staffing Office, UMDS, St Thomas's Hospital, Lambeth Palace Road, London SE1 7EH, quoting reference STH/VIR/151. Closing date: 21st March 1987. (2053)A

UNIVERSITY OF QUEENSLAND
Brisbane, Australia

LECTURER IN ZOOLOGY

Should have a higher degree in biology with demonstrated expertise and teaching experience in the fields of population ecology and genetics. Responsible to the head of Department of Zoology for the organising and teaching of undergraduate courses in basic and population genetics, and participating in the teaching of other areas in Zoology. Some background in vertebrate biology desirable. Expected to develop own research areas and to supervise postgraduate projects in Zoology. Appointment as soon as possible in 1987.

Salary per annum: $A27,859 - $A36,600.

Additional information and application forms are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Closing date 28 February 1987 or as soon as possible thereafter. (Ref: 01387).

Equal Opportunity in Employment is University Policy.

(W3814)A

M-Scan
Mass Spectrometry Consultants and Analysts

M-SCAN is a dynamic young Company with a record of pioneering new analytical methods in Bio-polymer research and analysis. Our Biotechnology Consultancy Services include the structural characterisation of *peptides, proteins, carbohydrates, glycoproteins* and *glycoconjugates* by both classical and mass spectrometric techniques. We are seeking to expand our Biochemistry Division with the following new appointments.

UK LABORATORIES

1. CARBOHYDRATE/GLYCOPROTEIN CHEMIST OR BIOCHEMIST

Requirements:
- Experience in carbohydrate, protein and/or glycoprotein chemistry.
- Willingness to learn new mass spectrometric methods.
- Ability to communicate scientific reports in a clear and concise manner, to organise work and meet deadlines.

2. GRADUATE BIOCHEMICAL ASSISTANT

Requirements:
- A degree or equivalent in a Biochemical Science.
- Experience of modern biochemical methods including HPLC, spectrophotometry and biopolymer purification techniques would be an advantage.

Applicants should send a c.v. and the names of two referees, in confidence, to Dr F. Greer, M-Scan Limited, Silwood Park, Sunninghill, Ascot, Berks. SL5 7PZ by 16th Mrch 1987.

U.S.A.

U.S.A. MARKETING EXECUTIVE

M-Scan Inc. is seeking an American Marketing Executive to actively expand our client base for M-Scan's unique Services and Hardware. This is an opening for a young, dynamic person seeking an opportunity to participate in an exciting new venture. The applicant should be responsible and self-motivated, and have several years of sales and marketing experience in the chemical, pharmaceutical or biotechnology industries. For further details, write to Dr H.R. Morris, M-Scan Inc (U.S.), P.O. Box 5368, Wilmington, Delaware 19808 U.S.A. (2081)A

FACULTY POSITION AVAILABLE

The University of California, Berkeley, has an opening, effective July 1, 1987, in the Department of Mechanical Engineering for a tenured position at the Associate or full Professor level, in the area of applied aerodynamics. Candidates should have a doctoral degree, a strong academic background in aerodynamics, and some background in the application of aerodynamics to design the performance of flight vehicles.

Send biography and the names of four references by March 31, 1987 to: **Applied Aerodynamics Search Committee, c/o Chairman, Department of Mechanical Engineering, University of California, Berkeley, California 94720. The University of California is an Equal Opportunity, Affirmative Action Employer.** (NW383)A

CHAIRMAN
DEPARTMENT OF BIOLOGY

Princeton University is seeking applicants for the position of professor and chairman of the Department of Biology. This department has investigators working in the areas of developmental and cell biology, ecology, evolution, and behaviour. Close interactions are maintained with the Departments of Molecular Biology and other science departments. The chairman will have the opportunity to recruit new faculty and is expected to play a central role in the future development of the department and the life sciences program at the university. It is also expected that the chairman will provide strong administrative leadership to the department, maintain an active research program and carry out teaching responsibilities.

Please send curriulum vitae with names of three references to: **Dr. Arnold J. Levine, c/o Laima Cole, Department of Biology, Lewis Thomas Laboratory, Princeton University, Princeton, NJ 08544.** ***Princeton University is an Equal Opportunity/Affirmative Action Employer, M/F.*** (NW491)A

UNIVERSITY OF CAPE TOWN

Professor/Associate Professor/ Senior Lecturer/Lecturer

in the Department of Botany

Applications are invited from all suitably qualified Botanists for one of the above vacant posts. No particular botanical field is specified, but the Department particularly wishes to strengthen teaching and research in physiological and evolutionary ecology.

The salary ranges are: Professor: R37 482-R45 069; Associate Professor: R31 782-R38 919; Senior Lecturer: R29 052-R36 045; Lecturer: R18 999-R31 782 per annum, with an annual bonus and attractive staff benefits.

Applicants should submit a full curriculum vitae and the names and addresses of three referees not later than 20 March 1987 to the Registrar (Attention: Appointments Office DB1187), University of Cape Town, Private Bag, Rondebosch 7700, Republic of South Africa.

Further information may be obtained from the above or from the Secretary, SA Universities Office, Chichester House, 278 High Holborn, London, WC1V 7HE.

(W3804)A

The University's stand against apartheid and all racially discriminating legislation is on record. Information on this as well as on the University's policy not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race or religion is obtainable on request.

Bates Wells Recruitment CT 066R

MRC CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow
Middlesex HA1 3UJ

crc

NON-CLINICAL SCIENTIST

Applications are invited from a postdoctoral scientist to participate in a project on acute inflammatory lung disease with particular reference to the involvement of oxygen derived free radicals.
It is hoped that the successful applicant will participate in other current research within the department which includes the measurement of drug concentrations and investigations of their toxic side effects.
This is a three year appointment with a salary at an appropriate point on the scales for university non-clinical academic staff.
Further details and applicatiion forms are available from The Personnel Dept. 01-864-5311 EXTN. 2673, quoting Ref: 101/1/3772.
Closing date for completed applications, 6th March 1987.
Medical Research Council is an Equal Opportunityu Employer.

(2098)A

IMPERIAL CANCER RESEARCH FUND

Molecular Geneticist

required by the ICRF Tumour Immunology Unit based at University College London to join the Hon. Director, Professor N.A. Mitchison. The Unit is concerned primarily with T lymphocyte function, and the scientist would be expected, with appropriate support, to develop own programme of research.

The appointment on a 6 year tenure-track Research Scientist level has a salary range of £14,175 to £17,700. Please quote ref: 57/87

Applications with full CV and names and addresses of three referees should be sent to the **Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX.** Closing date for applications 13th March 1987.

(2091)A

THE UNIVERSITY OF WESTERN AUSTRALIA

Perth

LECTURER (FIXED TERM)

(Ref: A02/87)

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a position as lecturer in Biochemistry. The appointment will be for an initial period of 3 years and may be extended. The appointee will be required to teach general biochemistry, including molecular biology, to undergraduate medical, dental, science and agricultural students, and to participate in the training and supervision of Honours and postgraduate research students. The appointment will be expected to pursue research either in one of the established research groups in the Department or as an individual. There are good research facilities available in the areas of intermediatry metabolism, biochemical endocrinology and developmental molecular biology. The appointee will be expected actively to seek funding for maintenance and special equipment from external sources. Applicants should have a PhD, a promising record of published research and some experience of university teaching. Preference will be given to candidates trained in basic biochemistry with experience in Molecular biology.

Further information can be obtained from the Head, Department of Biochemistry (09) 380 3325.

Salary Range: $A27,859—$36,600 p.a. (with $A5,223 p.a. loading if appointee has a medical degree).

Benefits include superannuation, fares to Perth for appointee and dependent family and removal allowance. Conditions of employment will be specified in any offer of appointment which may be mad as a result of this advertisement.

Written applications, stating full personal particulars, qualifications and experience and the names and addresses of three referees, should reach the Appointments Officer, University of Western Australia, Nedlands, Perth, Western Australia 6009, by 24 March 1987.

Equal employment Opportunity is University policy.

(W3811)A

THE QUEEN'S UNIVERSIT OF BELFAST

POSTDOCTORAL RESEARCH ASSISTANT IN CELLULAR IMMUNOLOGY

Multiple Sclerosis Research Laboratory

This position is funded by Actio MS for 3 years initially, to invest gate immunological function in th human CNS. The successful appl cant will contribute to an extensi programme of MS research an will collaborate with other men bers of the research group, i volved in histopathology, mole ular biology, electron microscop and immunocytochemistry. Ap plicants should preferably hav experience in cellular immunolog including cell culture. Furthe particulars may be obtained fro Professor Ingrid V. Allen, Neuro pathological Centre for Researc into Multiple Sclerosis and Allie Diseases, Institute of Patholog Grosvenor Road, Belfast, BT1 6BL. Please quote ref. 87/A Salary range: £8,022—£10,866.

RESEARCH ASSISTANT

DEPARTMENT OF APPLIED MATHEMATICS AND THEORETICAL PHYSICS

This post is funded by SERC fo three years, to maintain and de velop a large atomic data-bank This is a joint project between th University and the SERC Dares bury Laboratory. The researc will make extensive use of departmental VAX 11/750 and th large CRAY-XMP 48 super com puter which is located at the SER Rutherford Laboratory.

Applicants must have a go Honours degree and preferab PhD in physics, chemistry, applie mathematics or computer scienc and research experience in th development and use of larg scientific programs. A knowledg of atomic physics or astrophysic would be an advantage. Pleas quote ref. 87/ B. Salary range £8,022—£9,000.

Initial placing on salary range depends on age, experience an qualifications. Applications, in cluding curriculum vitae and th names and addresses of tw referees, should be sent to th Personnel Officer, The Queen' University of Belfast, BT7 1NN.

(2073)A

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MRC

MEDICAL RESEARCH COUNCIL CLINICAL PHARMACOLOGY UNIT

BIOCHEMICAL PHARMACOLOGIST

Applications are invited for a short term non-clinical scientific post in this MRC Unit, tenable for 3 years in the first instance. Candidates with some postdoctoral experience will be preferred, but those who are immediately postdoctoral will also be considered.

The successful applicant will be required to study the cellular and subcellular mechanisms by which cellular pharmacological adaptive responses occur, particularly in relation to the sodium potassium-ATPase. Knowledge of cell or tissue culture methods, radio-isotope techniques, enzyme and receptor assays, and kinetics will also be required. The work will progress to the study of the genetic control of adaptive responses. The programme has a strong clinical bias and will involve the investigation of human tissues.

Remuneration will be at an appropriate point on the scales for University non-clinical academic staff. Further information and an application form may be obtained from Professor D G Grahame-Smith, MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford, OX2 6HE, (Oxford (0865) 241091), to whom applications, including a full curriculum vitae and the names and addresses of two professional referees, should be sent by 9 March, 1987. (2038)A

RESEARCH ASSOCIATE

required to investigate the biophysics of antibody-antigen interactions. Must have at least two years training in protein crystallography and molecular immunology. This training must include the following: protein purification, isolation and crystallisation, collection of X-ray crystallographic data, the use of synchrotron radiation and two-dimensional nmr methods. Able to use interactive computer graphics and the techniques of molecular replacement, density modification and crystallographic refinement. Ph.D. in Biophysics and two years training required. Job and interview site: La Jolla, CA. Salary: $35,000/yr. 40 hrs/wk. Send this ad and your resume to Job # HO0273, PO Box 9560, Sacramento, CA 95823 - 0560 not later than 3/6/87. (NW477)A

POSTDOCTORAL/ RESEARCH ASSOCIATE

Position available immediately to study gene expression in Alzheimer's disease. Candidates must have a background in molecular genetics and skill in recombinant DNA techniques. Please send curriculum vitae and two references to:

D.R. McLachlan
Department of Physiology
Medical Sciences Building
Room 3318
University of Toronto
Toronto, Ontario
Canada M5S 1A8

(NW486)A

POSTDOCTORAL RESEARCH ASSOCIATE

Position available to do research on the molecular basis of photoadaptation in eucaryotic algae, as part of a collaborative effort between the Department of Applied Science and the Biology Department at Brookhaven National Laboratory. Candidates must hold a recent doctorate in plant or algal biochemistry, or physiology. A background in molecular biology is highly desirable.

Send curriculum vitae and names of three references to: **Dr. P.G. Falkowski, Department of Applied Science, Building 318, Brookhaven National Laboratory, Associated Universities, Inc., Upton, L.I., NY 11973.** *Equal Opportunity Employer m/f.*

(NW494)A

Sandoz Ltd., a leading Swiss-based international company with a strong commitment to pharmaceutical research, is offering a

Postdoctoral Position

in their newly created Biotechnology department. The successful candidate will be an ambitious PhD in molecular biology with interest in the regulation of eukaryotic transcription.

The position is available immediately for a period of two years.

Applications with curriculum vitae should be sent to:

SANDOZ

Sandoz Ltd.,
Personnel Department,
Ref. 7801
attn.: Mr R. Zbinden, P.B.
CH-4002 Basle/Switzerland

(W3805)A

POSTDOCTORAL TRAINING IN PLANT MOLECULAR BIOLOGY, MOLECULAR BIOLOGY, BIOCHEMISTRY AND GENETICS

The Plant Gene Expression Center (PGEC) in Albany, California, sponsored by USDA's Agricultural Research Service and the University of California, Berkeley, invites applications from qualified candidates with Ph.D. degrees for ten postdoctoral positions. The PGEC is an interdisciplinary program designed to provide plant agriculture with the technology to maintain and enhance the quality and productivity of the agricultural product.

The PGEC programs include: Auxin-regulated gene expression (A Theologis), maize transposable elements (B. Baker); monocot transformation (M. Fromm); gene expression in transgenic plants (D Ow), developmental mutants of maize (S. Hake); flower development (S. McCormick); and phytochrome regulation (P. Quail) Candidates should be familiar with molecular biology, biochemistry, or genetics Salary is commensurate with experience with a range of $22,000 $27,000. For information on the research program and/or positions, contact: Gerald G. Still (415) 486 3662. To obtain Federal application forms, contact Patricia Roberts (301) 344 3138, USDA, Agricultural Research Service, Room 405, Building 003, BARC-W, Beltsville, Maryland 20705.

An Equal Opportunity Employer

(NW468)A

FACULTY POSITIONS IN MOLECULAR & CELLULAR BIOLOGY

University of Arizona

As part of a major expansion, the Department of Molecular and Cellular Biology at the University of Arizona expects to hire one or more new tenure track faculty members this year. Preference will be given to applicants with research experience in molecular or genetic studies of developmental mechanisms, although outstanding candidates in other areas will be considered. We are particularly interested in junior candidates, although appointments at higher levels will be considered for outstanding individuals. Successful candidates will be expected to establish an excellent research program, as well as teach at the graduate and undergraduate levels.

Send curriculum vitae, 1–2 page statement of research interests, and have three supporting letters sent to: **Dr. Danny Brower, Search Committee Head, Department of Molecular and Cellular Biology, BioSciences West Bldg. Room 308, University of Arizona, Tucson, AZ 85721, USA.** Applications will be considered from March 15, 1987, until the positions are filled.

The University of Arizona is an Equal Opportunity/Affirmative Action Employer. (NW493)A

Associate or Full Professor of Pathology

THE SCHOOL OF VETERINARY MEDICINE
UNIVERSITY OF CALIFORNIA, DAVIS

is seeking applications for an Associate or Full Professor of Veterinary Pathology. Candidates must have a D.V.M. (or equivalent) and a PhD with advanced training in pathology. Preference will be given to persons with board certification by the American College of Veterinary Pathology. Candidates should have established a record as principal investigator of an extramurally-funded research program relating to the pathogenesis of disease at the cellular or molecular level. The candidate will participate in departmental teaching programs at the professional (D.V.M.) and postgraduate levels. The appointee will be expected to make an important contribution to the research programs of the department. The salary is commensurate with qualifications, experience and rank at appointment The starting date is July 1, 1987 with a deadline for receipt of applications being March 15, 1987 or until filled.

Applicants should send curriculum vitae, and the names of at least three references to **Dr D.L. Dungworth, Chairman, Search Committee, Department of Pathology, School of Veterinary Medicine, University of California, Davis, California, 95616.**

The University of California is an Affirmative Action/Equal Opportunity Employer. (NW448)A

THE INSTITUTE OF BIOMEDICAL SCIENCES, ACADEMIA SINICA, TAIWAN

is now offering

SENIOR OR JUNIOR SCIENTIST

positions in all areas of basic and clinical sciences, including biochemistry, biophysics, molecular genetics, cell biology, microbiology, immunology, physiology, pharmacology, pathology and epidemiology. Emphasis will be on those related to cancer, infectious and cardiovascular diseases, as well as neurosciences. Full operation of the Institute starts in 1987 with new building and excellent facilities. There will be close collaboration with the newly established Central Laboratory of Molecular Biology, and an opportunity to work with the Clinical Research Centers in major medical centers in Taipei. Send curriculum vitae and three recommendation letters to: **Dr Cheng-Wen Wu, Department of Pharmacological Sciences, SUNY Stony Brook, Stony Brook NY 11794.** *SUNY Stony Brook is an affirmative action-equal opportunity educator and employer. AK #7-A.* (NW479)A

UNIVERSITY OF EAST ANGLIA
Norwich

RESEARCH ASSOCIATE

required full time by the School of Biological Sciences, outposted at the Anglian Water Authority's Norwich fish farm, to work on experimental fish rearing. The appointment will be for 3 years with salary on the 1B scale, £7,055—£9,495 per annum (under review). Applicants should have experience of working with freshwater fish and possess a clean driving licence.

Applications, together with a curriculum vitae, and the names and addresses of two referees should be sent to Dr R. Linfield, Anglian Water Authority, Ambury Road, Huntingdon, Cambs. PE18 6NZ, within two weeks of the publication of this notice. (2067)A

POSTDOCTORAL POSITION

Cellular Immunologist

to work on studies concerned with neuroimmunomodulation. A good background in cellular immunology is required, and some experience in endocrinology would be valuable. Send c.v. and 3 references to: **Ronald Glaser PhD, Department of Medical Microbiology and Immunology, The Ohio State University, College of Medicine, 333 West 10th Avenue, Columbus, Ohio 43210.** (NW471)A

MENARINI MANAGEMENT POSITION

Menarini a leader in pharmaceutical research is seeking for an experienced highly qualified scientist to fill a challenging top management position in its research department. The successful candidate will be responsible for coordination of research programs at Menarini as well as its related companies and the development of relative research centers. He will be assisted, whenever necessary, by internationally recognized experts, and free to select his collaborators. Adequate means will be provided to carry out research programs.

The research centers are located in Florence and Pomezia (Rome). Headquarters are located in Florence.

An attractive salary is offered commensurate to qualification and experience.

Menarini will also consider the hiring of qualified young scientists with experience in cardiovascular, CNS, endocrine, respiratory or genito-urinary areas.

Qualified candidates are requested to submit their curriculum vitae and a list of recent publications to: Sergio Gorini, MD, A Menarini sas Pharmaceutical Industries, Via Sette Santi, 3, 50131 Florence, Italy. (W3817)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MEDICINE

(WGH)

RHEUMATIC DISEASES UNIT

CELLULAR IMMUNOLOGIST/ BIOCHEMIST

Applications are invited from postdoctoral cellular immunologists/ cell biologists/molecular biologists/ biochemists to join a cytokine research group investigating the role of Interleukin 1, tumour necrosis factor and other cytokines in the pathogenesis of rheumatic diseases. Funding is for three years from a five-year ARC Programme Grant, with the salary on the 1A scale (£9,495–12,780 pa) pending 1986 revision.

Further information may be obtained from Professor G Nuki or Dr G W Duff, Rheumatic Diseases Unit, Northern General Hospital, Ferry Road, Edinburgh EH5 2DQ (telephone 031 332 2525).

Applications, including CV and names and addresses of two referees, to Professor Nuki by 12 March 1987. Please quote reference number 5440. (2093)A

MARIE CURIE RESEARCH INSTITUTE

RESEARCH ASSISTANT

(1A or 1B)

required to work with Dr H. Sturzbecher to study nuclear oncoproteins and their cellular targets. Hands-on experience in a range of laboratory techniques is essential, especially protein gel electrophoresis, immunoprecipitation, mammalian cell culture etc. Candidates would normally be expected to have a degree in a biological science but proven technical ability will be the most important selection criterion.

The appointment is for 3 years in the first instance. Salary level will be set according to age and experience.

Applications, including a curriculum vitae and the names of two referees, should be sent to Jean Marr, Marie Curie Research Institute, Oxted, Surrey, RH8 0TL, UK, telephone 0883-722306. (2056)A

IMPERIAL CANCER RESEARCH FUND

POSTDOCTORAL RESEARCH FELLOW

MEDICAL ONCOLOGY UNIT, EDINBURGH

Applications are invited for a postdoctoral research fellow to join a small group involved in the preclinical evaluation of new anticancer drugs. The project involves the study of mechanisms by which anticancer drug pharmacology can be manipulated to improve response, reduce toxicity and overcome drug resistance.

PhD in pharmacology, pharmacy, molecular biology, cell biology or biochemistry. Salary range: £9,830 to £12,205.

Enquiries to Dr Cummings, ICRF Edinburgh Medical Oncology Unit (031 332 2471). Full c.v. and names and addresses of 3 referees should be sent to Ms S.M. Hurley, Personnel Officer, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX quoting ref: 58/87. (2064)A

POSTDOCTORAL POSITION

in Developmental Neurobiology available immediately for research on axonal growth and target interaction in the visual system of fish. Salary: 31.000,– DM/year. Send curriculum vitae and names of referees to: **Dr. C. Stuermer, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, P.O. Box 21 09, D-7400 Tuebingen, FRG.** (W3818)A

THE OPEN UNIVERSITY

FACULTY OF SCIENCE

RESEARCH ASSISTANT

Applications are invited for the post of research assistant in the Department of Earth Sciences. The postholder will develop techniques for the analysis of rock samples for the platinum group elements by graphite furnace atomic absorption spectrometry.

Applicants are likely to be recent graduates in geology or chemistry with a strong interest in techniques in analytical chemistry applied to rock analysis.

The post is available for one year initially. Salary will be within the 1B scale for research and analogous staff: £7,055—£9,495 p.a. (under review).

Application forms and further particulars are available from Mrs B. Woodall, (Post 5171/3), Faculty of Science, Wimpey II, The Open University, Walton Hall, Milton Keynes, MK7 6AA, or telephone 0908-653993; there is a 24-hour answering service on 0908-653868.

Closing date for applications 2nd March. (2054)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ANATOMY

RESEARCH ASSISTANT

Applications are invited for the above post for a project on tendon repair funded by the Scottish Home and Health Department. The project, for two years, will involve surgical techniques together with light and electron microscopy. Applicants must have or expect to obtain an upper second class degree in a relevant biological science, and would be expected to register for a higher degree (MSc).

Starting salary £7,055 p.a. on 1B Scale for Research and Analogous Staff (under review).

Further particulars and applications forms available from The Personnel Office, The University, Regent Walk, Aberdeen, AB9 1FX, (tel 0224 480241 ext 5351) with whom applications (2 copies) should be lodged by 6 March 1987. (Ref WS/017). (2070)A

Please mention

nature

when replying to these advertisements

ROWETT RESEARCH INSTITUTE

Bucksburn, Aberdeen,
AB2 9SB

BIOCHEMIST/ ENDOCRINOLOGIST

or studies on skeletal growth)

)plications are invited from biochemists or endocrinologists for a nior position within the Division Biochemistry to lead a team vestigating the control of eletal growth. The person pointed will have a proven search record which is likely to in an area related to the action local and systematic growth ctors on bone and cartilage cells, d the response of these cells to rmonal stimuli.

This work will be carried out thin the Skeletal and Conctive Tissue Metabolism Group d there will be close links th projects involving collagen etabolism and Ca/P homeostatic echanisms.

Appointment will be made at ther Senior Scientific Officer Grade 7 level within the underted salary ranges in accordance th qualifications and experice.

Salary range: SSO £10,558 to 3,762; Grade 7 £14,318 to 9,465. The Institute is an equal portunities employer and a n-contributory superannuation heme is in operation.

Applications in writing together ith a full cv and the names and ldresses of two referees should submitted to the Secretary of e Institute from whom further tails may be obtained. Tel: 0224 2751. Please quote post ref: 48. Closing date: 5 March 1987.

(2034)A

UNIVERSITY OF MANCHESTER

DEPARTMENT OF CELL AND STRUCTURAL BIOLOGY (IMMUNOLOGY GROUP)

LECTURERS (TWO)

pplications are invited from ostdoctoral graduates with a esearch interest in any aspect of nmune regulation to join the cademic staff of an expanding nd progressive department.

The Lecturers will be required conduct an active programme of esearch and to teach Science and Medical Students at undergraduate and postgraduate levels. alary range p.a. (under review): 8,020—£15,700. Superannuaion.

Informal enquiries to Professor .V. Hutchinson, Immunology roup, Department of Cell and tructural Biology, the University, Manchester M13 9PT. Pariculars and application forms returnable by April 3rd) from the egistrar, the University, Manhester M13 9PL. Quote ref. 16/ 7/NA.

(2048)A

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

DIVISION OF VIROLOGY

POSTDOCTORAL RESEARCH ASSISTANT

to work on the genetic response controlling normal cell proliferation. The project will be integrated with ongoing studies on the expression of proteins encoded by proto-oncogenes. The successful candidate will join an active research group; expertise in genetic engineering and related technologies an advantage but not essential.

The post (3 years) is funded by the Wellcome Trust and may be activated immediately; salary up to £9,495—£10,375 (under review). Candidates must be under the age of 32 years (science graduates) or 35 years (medical graduates) and have studied/ worked in a University/Hospital in the UK over the past three years.

Applications (with curriculum vitae and the names and addresses of two referees) should be sent to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. Please quote 9412. Further details from Dr Jo Milner (0223 336916).

(2037)A

UNIVERSITY OF LONDON

CHAIR OF CHEMICAL PATHOLOGY AT KING'S COLLEGE, LONDON

The Senate invite applications for the above Chair. Applications (10 copies) should be submitted to the Teachers' Section (N), University of London, Senate House, Malet Street, London WC1E 7HU, from whom further particulars should first be obtained.

The closing date for receipt of applications is 20 March 1987.

(2065)A

UNIVERSITY OF EDINBURGH

TEMPORARY LECTURER IN ZOOLOGY

(Applied Entomology)

Applications are invited for a temporary Lectureship in Zoology for one year only from 1 October 1987. Placement on the scale of £8,020 to £15,700 will be according to age and qualifications. The main teaching duties are in applied entomology. Further particulars can be obtained from The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS with whom applications by letter (2 copies) including a curriculum vitae and the names of two referees should be lodged not later than 31 March 1987. Please quote reference number 1283.

(2059)A

TRANSPLANTATION IMMUNOLOGIST

The Cleveland Clinic Foundation invites applications for a full-time position involving a primary appointment in the Department of Immunology & Cancer and a joint clinical appointment. Interested applicants must have an MD and/or PhD degree with considerable postdoctoral experience in basic transplantation immunology. Candidates must be established investigators with evidence of high quality independent research, and a consistent history of external funding support. Essential qualities of the successful applicant are the ability to develop a research program bridging basic laboratory investigation with relevant problems in transplantation science, and a willingness to interact with basic scientists as well as transplant clinicians. Please submit a letter of application, curriculum vitae, and research interests to:

Andrew C. Novick, M.D.
Chairman, Department of Urology

THE CLEVELAND CLINIC FOUNDATION

The Cleveland Clinic Foundation is an Equal Opportunity/Affirmative Action Employer.

(NW473)A

UNIVERSITY OF LONDON

ROYAL POSTGRADUATE MEDICAL SCHOOL

with

HAMMERSMITH AND QUEEN CHARLOTTE'S SPECIAL HEALTH AUTHORITY

SAFETY OFFICER

Applications are invited for the full time post of Safety Officer for the Royal Postgraduate Medical School and Hammersmith and Queen Charlotte's Special Health Authority.

Applicants should have previous experience of safety within a laboratory and/or hospital context. Candidates should be suitably qualified, preferably in a relevant scientific discipline.

Salary on grade II for Administration in Universities, currently £12,280 to £15,700 plus £1,393 London Allowance a year (Salaries under review).

Application forms and further particulars may be obtained from the Personnel Officer, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting reference EE/SO. Closing date: 26th February 1987.

(2039)A

SCOTTISH UNIVERSITIES RESEARCH AND REACTOR CENTRE

East Kilbride

TECHNICIAN GRADE 7

(£9713–£11,005)

A senior technical post is immediately available within a 17 strong research group which applies a wide range of mass spectrometry facilities to geological research, mainly in collaboration with Scottish university departments. The present vacancy is tenable until September, 1988, being funded by an NERC research grant In the past the grants and hence the post, have been successively renewed and thus the present appointee is taking early retirement after 20 years in the post.

Applicants should have an HNC or equivalent qualification and have at least ten years experience (including training). Experience in electronics and computer programming is required. Vacuum system and mass-spectrometric experience are desirable but not essenital. The post may involve some R and D in collaboration with instrument manufacturers.

Applications in writing giving details of age, qualifications and experience should be submitted to the Personnel Officer (Non-Academic), University of Glasgow, Glasgow G12 8QQ not later than 20th February, 1987.

(2084)A

FRIEDRICH MIESCHER-INSTITUT

Applications are invited for the position of a

GROUP LEADER IN PLANT BIOLOGY

Candidates should have a strong research background in Plant Sciences at the cell and/or molecular level, as well as a demonstrated ability to develop an independent research programme and to lead a group.

The candidate will be expected to carry out research in an area contemplating the work of five other group leaders: Witek Filipowicz (gene expression and RNA processing), Barbara Hohn (genetic flux in plants), Thomas Hohn (molecular plant virology), Patrick King (a genetic approach to plant hormones), and Frederick Meins (plant development). The groups each consist of postdoctoral fellows, graduate students and technical staff, and occupy independent laboratories.

We would prefer to make the appointment at the level of a senior group leader, but an appointment at a more junior level is not excluded.

A curriculum vitae and a list of publications should be submitted before March 31st, 1987 to: **Search Committee (Plant Group Leader), Friedrich Miescher-Institut, PO Box 2543, 4002 Basel, Switzerland.**

(W3794)A

UNIVERSITY OF BATH

School of Biological Sciences

AND

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF MICROBIOLOGY

RESEARCH ASSISTANT

Applications are invited for a position funded by SERC to study the role of cyclic AMP, GTP and adenylate cyclase in the regulation of proliferation of the yeast saccharomyces cerevisiae. The work will develop collaboration between Dr A E Wheals (Bath) and Dr J R Dickinson (Cardiff) and will involve biochemical analyses and recombinant DNA methodology. The position is available (for one year in the first instance) from 1 April 1987, at a salary of £7,055. The start could be delayed until 1 October.

Application forms from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting Ref No 87/29. Informal contact may be made with Dr A E Wheals (0225 826826, Ext 4278). Closing date 26/2/87. (2089)A

MEDICAL RESEARCH COUNCIL

Clinical Oncology and Radiotherapeutics Unit

RE-ADVERTISEMENT

Salary range correction

MOLECULAR BIOLOGIST

Short Term (3–5 Years)

SCIENTIFIC APP

This newly-created position is for a molecular biologist to join an existing team working on a range of projects aimed at improvement of cancer therapy. The work currently includes studies on the biology of human lung cancer, tumour markers, pharmacology of anti-cancer agents, mechanisms of drug resistance, detection of cellular oncogene products using flow cytometry, the role of DNA damage and repair in therapeutic response and hyperthermia as a treatment modality. The successful candidate would be expected to contribute to one or more of these projects but would also be encouraged to develop his or her own related interests. Experience in gene cloning techniques would be an advantage.

The position is available immediately. The post is tenable for 3-5 years on the MRC non-clinical scientific scale, salary range £9,495 — £15,700.

Applications in writing should be sent by 5 March 1987, with cv and the names of two Prof referees, quoting Ref: CO2 to: The Personnel Officer, Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH.

The medical research council is an equal opportunity employer.

(2100)A

QUEEN MARY COLLEGE

(University of London)

DEPARTMENT OF CHEMISTRY and

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

DEPARTMENT OF MEDICINE

Applications are invited for

TWO RESEARCH POSITIONS

for work supported by the MRC on dipyrrolic substances in bilirubin catabolism in collaboration with Professor R Bonnett and Professor B H Billing. The senior post is at the postdoctoral level for work on chemical aspects of the probem at Queen Mary College. The second post is for a graduate with biochemical experience and is tenabe at the Royal Free Hosipital, Hampstead. Appointments are for 1 year in the first instance, starting 1st April 1987 or as soon as possible thereafter.

Salary scales: £7,055 — £9,495 postgraduate, £8,020 — £12,780 postdoctoral, plus £1393 London Allowance.

Writen applications (2 Copies) with names of two referees to the Assistant Personnel Officer, Queen Mary College, Mile End Road, London E1 4NS, from whom further information may be obtained. Please quote Reference 87/21. (2088)A

ASSISTANTSHIPS

UNIVERSITY OF BRISTOL

Department of Biochemistry

POSTDOCTORAL RESEARCH WORKER

Applications are invited for a three year Research Assistantship to work on a Medical Research Council funded project on the structure and molecular genetics of cytoskeleton-associate human red cell membrane protein antigens. The project involves collaboration between Dr M J A Tanner, (Department of Biochemistry) and Dr D J Anstee (Regional Blood Transfusion Centre, Bristol). Applicants should have a background in Biochemistry or related subjects. The project will involve the use of immunological and recombinant DNA methods. Experience in recombinant DNA methods would be desirable but is not essential and training in the area will be given if needed. Applicants should already have a PhD or expect to receive one shortly, and the salary is on the Range 1A scale.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to: Dr M J A Tanner, Department of Biochemistry, University of Bristol, Bristol BS8 1TD.

(2078)P

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLIGY

A RESEARCH ASSISTANTSHIP

is available in the laboratory of Dr W J Ingledew, department of biochemistry and microbiology. The post (for approximately 2 years, on 1B salary scale £6,600 to £7,055 per annum, under review) is part of an SERC funded project for a study of the biochemistry of sulphur and ferrous iron oxidation by thiobacillus ferroxidans and is suitable for a graduate in biochemistry, chemistry or microbiology. A case studentship is also available.

Further particulars may be obtained from Dr W J Ingledew, department of biochemistry and microbioogy, The University, College Gate, St Andrews, Fife (Tel 0334 76161, Ext 270) to whom applications with the names of two referees should be sent to arrive not later than Friday 6th March, 1987. (2087)P

continued on page 20

FELLOWSHIPS

UNIVERSITY OF EDINBURGH

DEPARTMENT OF PRECLINIC VETERINARY SCIENCES

POSTDOCTORAL RESEARCH FELLOWSH IN NEUROSCIENCE

A Postdoctoral Research Fello required to join a MRC Prograı Grant team studying the anaton and functional properties of idı fied neurons in the spinal Experience of in vivo neurophy logical techniques is required, ı erably including intracellular as as extracellular microelect methods and ionophoresis. The will be available from 1st May, and is funded to 31st Decem 1988 on the grade 1A Scale (£8 — £12,780) with a possibiliy o newal for a further period of up years. Further information ma obtained from, and applications cluding a cv and the names of referees) should be sent to: Prc sor A G Brown, Department of clinical Veterinary Sciences, Uni sity of Edinburgh, Summerl Edinburgh, EH9 1QH.

Plese quote reference No 543

(2092)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLO AND PHARMACOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited fo research fellowship funded SERC for studies of α-adre ceptor mechanisms in the cen nervous system.

The project is concerned v the assessment of α-adrenocep stimulated second messen formation in the brain. Experie of inositol phospholipid hydrol and/or cyclic AMP measureme would be an advantage. appointment is for three ye beginning as soon after 1st A 1987 as possible, with salary on University Research 1A scale.

Further information from D.A. Kendall (0602 700111 3611) to whom applications w c.v. and names of two refer should be sent at Medical Schc Queen's Medical Centre, Notti ham NG7 2UH. (2076)E

continued on pag 17,18 &

nature

— the professionals choice

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH IN IOLOGICAL CHEMISTRY

postdoctoral research fellow-
ip is available funded by SERC
otechnology, for research into
pects of antibiotic production in
reptomycetes. The research will
volve enzyme purification, and
ne cloning, and would be suit-
le for persons with prior training
bio-organic chemistry or bio-
emistry. The post is available
mediately, for one year initially,
newable for a further two, salary
cording to age on the scale
,020—£9,495. Applications and
quiries to Dr J.A. Robinson,
hemistry Department, The Uni-
rsity, Southampton, SO9 5NH,
el. 0703 559122 Ext. 3598. Please
ote ref. no. 69/HFW/MB/
A. (2049)E

UNIVERSITY COLLEGE, DUBLIN

DEPARTMENT OF GEOLOGY

he Department invites applica-
ons from suitably qualified
dividuals for Department of
ducation Postdoctoral Fellow-
ips 1987-88. These are admin-
tered by the Department of
ducation and are tenable for a
eriod of two years with a possible
tension for a third year. Appli-
nts should hold the degree of
hD or have equivalent research
perience at the time of taking up
e award. They should be under
and preferably under 28 years
f age on March 31, 1987.
ppointment will be effective
om a date not earlier than
ctober 1, 1987. Salary, depen-
ent upon qualifications and ex-
erience, will be in the range
£8,050—IR£9,108 per annum.

The Department of Geology is
articularly interested in applica-
ons from persons with interests in
adiogenic isotope geochemistry,
uid inclusion studies and
ineralisation, carbonate petro-
gy and diagenesis, micropalae-
ntology or Irish Caledonian
eology, but applicants with other
search interests will also be con-
dered. Interested persons are
equested to contact Prof. M.J.
ennedy, Department of Geo-
gy, University College, Belfield,
ublin 4, Ireland for further par-
culars of the facilities and re-
earch opportunities in the
eology Department.

Application should be made
irectly to the Secretary, Depart-
ent of Education, (Head-
uarters Section 3), Marlborough
treet, Dublin 1, from whom
pplication forms and full particu-
rs of the fellowship scheme can
e obtained. The deadline for
eceipt of applications by the
epartment of Education is
arch 31, 1987. (2069)E

Sandoz Institute for Medical Research
Gower Place, London WC1

Post-Doctoral Fellowship in Cellular Neurobiology

Applications are invited for a post-doctoral fellowship, tenable for up to 2 years, in the Cell Biology group under the supervision of Dr R. M. Lindsay. The appointed candidate will join a project studying regulation of the biosynthesis and release of neuropeptides from nociceptive sensory neurones. As much of the work will entail the use of cultured cells, some tissue culture experience would be useful, but an interest in cellular neurobiology and strong biochemical background are more essential qualifications.

The post offers scope to collaborate in a multi-disciplinary study of the basic properties and functions of sensory neurons, studies which are linked towards the development of new analgesic drugs.

Salary will be in the range £11.000-£15,000 p.a. Applications, including the names of two referees, should be sent to Mrs M. C. Stuart,
Sandoz Institute for Medical Research, 5 Gower Place,
London WC1E 6BN, by
15th March 1987.

(2071)E

SANDOZ

UNIVERSITY OF QUEENSLAND
Brisbane, Australia

NATIONAL RESEARCH FELLOWSHIP PHYSIOLOGY AND PHARMACOLOGY

To work on ARGS funded project on reproduction in Australian flying foxes (genes Pteropus). Appointee will play a major part in the project including the development of radioimmunoassays to measure reproductive hormones and elucidate their role in the integration of the hypothalamic — pituitary — gonadal system. The project has been running successfully for three years. The department has an established breeding colony of grey-headed flying foxes. Qualifications: PhD or equivalent; research experience in reproductive biology and/or radioimmunoassay an advantage.

Salary $A24,013 - $A27,505.per annum. Superannuation not available. Appointment 3 years. Contact Dr Len Martin (07) 377 3128 for further information.

Applications detailing full information to the Director, Personnel Services, University of Queensland, St Lucia, 4067, Queensland, Australia. Please quote Ref No: 48386 and department concerned. Closing date 28 February 1987.

Equal Opportunity in Employment is University Policy.

(W3813)E

TOUR DU VALAT POSTDOCTORAL FELLOWSHIP IN ECOLOGY

Applications are invited for the second Tour du Valat postdoctoral fellowship in ecology. The fellowship, which is for two years, will be held at the Station Biologique de la Tour du valat, Camargue, France The Fondation Tour du Valat is an independent research organisation specialising in fundamental and applied research in the ecology of Mediterranean wetlands. This fellowship is offered within the general area of wetland ecology; preference will be given to candidates working on the ecology of submerged macrophytes, fish ecology or avian ecology. Further details may be obtained from the Scientific Director, Station Biologique de la Tour du Valat, Le Sambuc, 13200 Arles, France. The closing date for applications is 31.05.87. (W3810)E

AHFMR
RESEARCH FELLOWSHIPS IN THE BIOLOGICAL SCIENCES

The University of Calgary, Department of Biological Sciences, invites applications from highly qualified researchers in medically-related areas of the biological sciences. Successful candidates will be nominated for Scholarships awarded on a competitive basis from the Alberta Heritage Foundation for Medical Research. The Foundation provides a salary and an establishment grant to set up a laboratory Appointment will be at the assistant professor level with remuneration based on the salary scale at The University of Calgary (1986· $31,247 - $48,719). Awardees will be expected to devote at least 75 per cent of their time to research activities. Limited teaching duties and participation in departmental affairs will be required.

Applicants must hold the Ph.D. degree and have at least two years' postdoctoral experience. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.

Qualified individuals should send their curriculum vitae, a brief description of their research objectives, and the names of three referees prior to March 31, 1987 to:

Chairman, Scholarship and Fellowship Review Committee, Department of Biological Sciences, The University of Calgary, Calgary, Alberta, Canada T2N 1N4.

(NW485)E

POSTDOCTORAL RESEARCH FELLOWSHIPS

MOLECULAR BIOLOGY — MOLECULAR IMMUNOLOGY

☆ ☆ ★ ☆ ☆

We are looking for Postdoctoral Research Fellows to join Dr Mario Geysen's group working in a new era of drug development through the wide and systematic application of a unique peptide synthesis technology and concepts. Target areas cover virtually all therapeutic categories, e.g. hormone analogues, antiviral agents, vaccines, etc. A relevant PhD is essential with preference being given to candidates who have had one previous postdoctoral position.

These positions offer the prospect of independent research and publication will be strongly encouraged.

CSL is Australia's leading manufacturer of biologicals and has a strong commitment to Research and Development. The unique work in molecular immunology is conducted through Dr Geysen's group and has very significant levels of funding and autonomy; the work is already being progressed on many fronts and through important international collaborative projects.

We offer competitive salaries and travel and associated benefits for these 2 year appointments. Appointees will commence with us during the second half of 1987.

Please send a cv outlining your qualifications, description of scientific experience and the names of two referees, to Mr E. D. Eade, Personnel Director, Commonwealth Serum Laboratories, 45 Poplar Road, Parkville, Vic. 3052, Australia, by 16 March 1987. (W3754)E

CSL COMMONWEALTH
SERUM
LABORATORIES

NSERC
University Research Fellowship

The University of Calgary, Department of Biological Science
invites applications for research fellowships in its five division
Botany; Cellular, Molecular and Microbial Biology; Ecology; Bi
chemistry; and Zoology. Successful applicants will be nominate
for University Research Fellowships awarded in open cor
petition by the Natural Sciences and Engineering Researc
Council of Canada. Appointment will be at the assistant profess
level. Remuneration will be based on the salary scale at tl
University of Calgary (1986: $31,247—$48,719).

Applicants must hold the PhD degree and have some pos
doctoral experience. Only those who have a demonstrated abili
to carry out productive and independent research of a hig
calibre will be considered. NSERC Fellowships are open only
Canadian citizens or permanent residents of Canada.

Qualified individuals should send their curriculum vitae, a bri
description of their research objectives, and the names of thre
referees before March 31, 1987 to: **Chairman, Scholarship ar
Fellowship Review Committee, Department of Biologic
Sciences, The University of Calgary, Calgary, Alberta, Canac
T2N 1N4.** (NW484)A

POSTDOCTORAL RESEARCH FELLOWSHIP

to study gene transfer and expression available for a 2 year period beginning April 1, 1987. Applicants should have a PhD and research experience in biochemistry or molecular biology. The project will involve the analysis of expression of transferred human globin genes. Send c.v. and 2 letters of recommendation to: **Dr A. Keating, Oncology Research, Toronto General Hospital, 200 Elizabeth Street, Toronto, Ontario M5G 2C4.** (NW487)E

POSTDOCTORA
FELLOWSHIP
(Microbiology)

in the Ocular Microbiol
Laboratory, Department
Ophthalmology, Baylor Coll
of Medicine, Houston. To e
uate pathogenesis of eye in
tions associated with con
lenses. Previous experience
developing rapid detect
methods and/or evaluating
herence mechanisms. Send c
riculum vitae and refere
names to: **K.R. Wilhelmus, M.
Cullen Eye Institute, One Bay
Plaza, Houston, TX 77030.**
(NW476)

MARIE CURIE VISITING FELLOWSHIP PROGRAMME

The Marie Curie Foundation will provide Fellowships for ove
seas scientists to work in its Cancer Research Institute at Oxte
Surrey.

Situated 15 miles from London, near London-Gatwick airpor
mainline train services and motorways, this Institute is expanding i
commitment to studying various aspects of cellular and molecul
biology of human cancer. Current topics include structural and fun
tional analysis of the p53 nuclear oncoprotein, molecular basis
cellular senescence and immortalization, the control of gene expre
sion at the level of transcription and its deregulation in cancer cell
growth regulation and motility in melanocytes, the molecular biolog
of human malignant melanoma, transformation of human urotheli
cells herpes virus gene regulation, etc. There are extensive inte
national collaborations already in existence and we wish to er
courage their further development.

These fellowships will fund appointments for young scientis
and/or clinicians with established skills and interests relevant to th
above topics. Economy class travel and an appropriate academ
salary will be provided.

Applications, a detailed curriculum vitae and the names of thre
referees should be sent to **Jean Marr, Secretary to the Director, Mar
Curie Research Institute, Oxted, Surrey, RH8 0TL, U.K., telephon
0883-722306.** (2086)E

Fidia Research Foundatio
Fellowships in Neuroscien

B.B. Brodie
Fellowship in
Neurochemical
Pharmacology

G. Moruzzi
Fellowship in
Neurophysiology

A unique opportunity for two Italian M.D.s to continue or expand their basic research experience. The program of each fellowship is flexible and may be established at any United States research institution selected by the candidate and approved by the laboratory director.

These fellowships are open to M.D.s with two years of basic research experience who will not be older than 30 at the filing deadline. The deadline for applications is 15 March 1987. Applications should include a copy of Medical Degree (M.D.), a letter from the department head attesting to basic research experience for two years following the degree, two letters of support by [illegible] supervisors, and a letter from the director of the U.S. laboratory, selected by the candidate, who would make space available, supervision, and sufficient additional research support for the two year period. Proof should be given that research and training programs have been agreed upon by the applicant and the head of the host laboratory. Also please include proof of Italian citizenship.

Each award will consist of a yearly $20,000 stipend for the two years, and a round trip ticket to and from the laboratory selected by the candidate. Each fellowship will also include a $4,000 yearly contribution to the host laboratory to partially support research expenses including attendance at scientific meetings. Research and training programs will be agreed upon by the applicant and the head of the host laboratory.

Within three months of the termination of the fellowship, the awardee must have prepared a written presentation of the research program experience, and research results for [illegible] by the Scientific Advisory Board of the Fidia Research Foundation for eventual publication.

Applications should be sent to:
B.B. Brodie/G. Moruzzi Fellowships
Fidia Research Foundation
1775 K Street, N.W., Suite 801
Washington, D.C. 20006 (U.S.A.)

Decision on the Awards will be made by 30 June 1987 by the Board of Scientific Directors of the Fidia Research Foundation. Fellowships can be [illegible] any time after the award is made.

Fidia
Research
Foundation

Scientific Advisory Board

Dr. [illegible] - Chairman of the Scientific Board Fidia Research Foundation - Washington, DC 20006

Dr. Paul Greengard - The Rockefeller University Laboratory of Molecular and Cellular Neuroscience New York, NY [illegible]

Dr. Eric Kandel - Howard Hughes Medical Institute Columbia University - New York, NY 10032

Dr. Frank Standaert - Vice President and Dean Medical School of Ohio at Toledo - Toledo, OH [illegible]

Dr. Gerald Fischbach - Edison Professor of Neurobiology Washington University School of Medicine - St. Louis, MO [illegible]

Dr. Floyd E. Bloom - Division of Preclinical Neuroscience and Endocrinology Scripps Clinic - La Jolla, CA 92037

ANNOUNCEMENTS

SPECIAL TOPIC PROGRAMMES

Natural Environment Research Council

NERC's Special Topic scheme provides money to stimulate research in priority areas of the natural environmental sciences. In particular it promotes collaboration between academic departments, NERC research institutes and other bodies.

Six Special Topics are announced:

1. BASIN DYNAMICS — evolution and dynamics of sedimentary basins through an integrated approach involving sedimentology, tectonics, geochemistry and geophysics in collaboration with the British Geological Survey. £310k over 4 years. Contact Mr A Mayer.
2. OCEAN DRILLING PROGRAMME (ODP) — to support shore-based work arising from UK membership in ODP, including regional geological and geophysical studies, co-ordinating shipboard and onshore research, post-cruise synthesis studies, the construction and operation of instruments for innovative downhole experiments or logging. £1,090 over 4 years. Contact Mr E Nickless.
3. ENVIRONMENTAL RADIOACTIVITY — to increase research on transfer and uptake processes of radionuclides in the natural environment, in particular on aspects where shortcomings in understanding have been shown up by observations since the Chernobyl incident. £385k over 4 years. Contact Dr M Schultz.
4. BIOGEOCHEMICAL OCEAN FLUX STUDIES (BOFS) — BOFS is designed to study the biogeochemical processes influencing the dynamics of elemental cycling, particularly carbon, in the oceans, through inter-related research on air-sea exchange, primary production, particle production and fate and benthic fluxes. It will prepare the way for global monitoring of biological production by satellite observations of ocean colour. It is a pre-requisite to understanding biological control of climate. £1,545k over 5 years. Contact Dr P Williamson (0752 221371 — IMER Plymouth).
5. NORTH SEA PROJECT — fifteen months of dedicated ship time will be used to collect a new data base describing the seasonal cycle in the North Sea. This data base is needed for 3D modelling of physical processes leading to an advanced prognostic model of water quality and sediment movement for predicting the fate of pollutants in the North Sea. £780k over 5 years. Contact Dr J Huthnance (051653 8633 — IOS Bidston).
6. FINE RESOLUTION ANTARCTIC MODEL (FRAM) — using the advanced computing power of the CRAY XMP/45 supercomputer, FRAM aims to run and analyse an eddy resolving model to predict circulation in the Southern Ocean, in time for the World Ocean Circulation Experiment (WOCE). The project is a pre-requisite for exploiting the data from the ERS-1 ocean-observing satellite, due in 1990. £545k over 5 years. Contact Dr P Killworth (0865 274590 Robert Hooke Institute).

The announcement of opportunity for ODP is already available (closing date for research grant applications in 1987 are 6 March, 1 June, and 1 October). Specifications for the other five schemes are being prepared and will be publicised shortly. The officers named can be contacted for further information. For topics 1-3 contact: **Natural Environment Research Council. Polaris House, North Star Avenue, Swindon, Wiltshire SN2 1EU. Telephone 0793 40101.** (2090)G

ASSISTANTSHIPS continued

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited for a

SENIOR POSTDOCTORAL RESEARCH ASSISTANTSHIP

tenable for three years. The successful applicant will participate in a programme of theoretical study of charge-changing collisions, with the emphasis on the role of autoionisation.

Initial salary up to £9,495 per annum on Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent (quoting reference P4) by 27 February 1987 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (2046)P

STUDENTSHIPS

MRC CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow
Middlesex, HA1 3UJ

MRC RESEARCH STUDENTSHIPS

There exist a number of opportunites for PhD student
ships at this unique clinical research establishment. Can
didates holding or expecting to obtain a first class o
upper second class honours degree in an appropriat
subject are invited to apply for three year studentship
commencing in September/October 1987.

Projects are available in a wide variety of subject area
ranging from biochemistry to molecular biology, phar
macology and physiology. For further information, pleas
telephone the Personnel Office 01-864-5311 extensio
2348.

The Medical Research Council is an equal opportunit employer. (2075)F

JOHN INNES INSTITUTE

PhD STUDENTSHIPS

The Institute is offering eight studentships, starting in October 1987, for work on the following topics: molecular biology of plant cell infection by *Rhizobium*; molecular genetics of *Streptomyces* (antibiotic production, differentiation, genome analysis); storage protein production during seed development studied by *in situ* hybridisation; biosynthesis of fatty acids versus starch in pea seeds; cloning of pea glutathione reductase; plant proteins involved in virus promoter selection; molecular biology of RNA plant viruses; interactions between the plant plasma membrane and the cytoskeleton.

For full descriptions of the projects and application procedures contact Professor D.A. Hopwood, John Innes Institute, Norwich NR4 7UH (phone 0603 52571). (2068)F

UNIVERSITY OF ABERDEEN

MEDICAL RESEARC
STUDENTSHIPS

Applications are invited for
studentships for research witl
range of disciplines including
teriology, biochemistry, obst
and gynaecology, patho
physiology, pharmacology
surgery, within the facult
medicine. The value of the
dentships is similar to those o
research councils.

EAF and further details
the Clerk to the Faculty of
cine, University of Aber
Regent Walk, Old Aberdeen
1FX. Closing date for recei
completed applications: 13 M
(209

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF HUMAN METABOLISM AND CLINICAL BIOCHEMISTRY

POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited for postgraduate studentships tenable from 1 October 1987. The successful candidates would work in one of the following areas:

1. The regulation of the function of human bone cells in vitro.

2. The action of cytokines on connective tissue.

3. Interactions between intracellular signalling systems in pituitary and neural cells.

4. Neuroendocrine control i
hypothalamic-pituitary axis.

5. Studies on the mechani
action of growth factors.

The Department has exc
facilities for laboratory and c
work, with emphasis on hor
and neurotransmitter action,
mone secretion, calcium n
olism, connective tissue biolog
cancer research.

Applications, which
include a curriculum vitae a
names of two academic ref
should be sent to Dr B L B
Department of Human Meta
and Clinical Biochemistry,
Hill Road, Sheffield S10 2R
0742-766222, ext 3037) as s
possible.

An Equal Opportunity Emp
(20

JOHN INNES INSTITUTE

PLANT CELL BIOLOGY SUMMER SCHOOL

7-18 SEPTEMBER 1987

This course of practicals and lectures for advanced research workers will cover the isolation and characterisation of macromolecules, in situ hybridisation, protoplast techniques, the subcellular localisation of antigens by immunofluorescence and immunogold methods, electron microscopy including cryotechniques, deep etching, negative staining and image analysis.

The Course Organisers are: K. Roberts, D. R. Davies, C. W. Lloyd, G. J. Hills, N. Brewin and A. Maule.

Invited Lecturers include: D. H. Northcote, A. Trewavas, H. Dickinson, R. Selvendran, D. Bowles, G. Galfre, J. Beezley, J. Ellis, R. Perham, P. Shaw, and M. Akam.

The Course Fee, which will include full board and accommodation and the 2 week course fee, is £500.

Enrolment: 15 places.

Further information and application forms from:

Dr. K. Roberts,
Department of Cell Biology,
John Innes Institute, Colney Lane,
NORWICH NR4 7UH.

Deadline for application — 21 April, 1987. This school is mounted with support from the Company of Biologists and from the Nuffield Foundation.

(2042)C

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BIOTECHNOLOGY

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Versailles, 25–26th May 1987

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SCIENTIFIC PROGRAM

I – LECTURES
- **A NEW EXPERIMENTAL MEDICINE AND EDUCATIVE MEANS IMPROVEMENT.**
- **MATHEMATICAL AND PHYSICAL MODELS.**
- **EVALUATION METHODS OF IN VITRO TOXICITY.**
- **BIOCOMPATIBILITY, IMMUNOPHARMACOLOGY AND IMMUNOCHEMIOTHERAPY.**

II – POSTERS
- **DISCUSSION OF POSTER COMMUNICATION ON SYMPOSIUM THEME.**

REGISTRATION FORM

(to return to *l'A.F.I.B.I.O.V. 90, J.P. Timbaud 75011 PARIS).*

First name/Prénom ....

Occupation/Profession ....

Position/Fonction ....

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2 °) STUDENTS:
FF 300 per person, say FF x .... = FF ....
TOTAL FF ....

continued on page 22

SEMINARS & SYMPOSIA

Thirteenth EMBO Symposium
MOLECULAR BIOLOGICAL ASPECTS OF NEUROBIOLOGY
14 – 17 September 1987
EMBL, Heidelberg. F.R.G.

Invited speakers include:
Y.-A. Barde (Martinsried), E.A. Barnard (Cambridge), M.J. Berridge (Cambridge), H. Betz (Heidelberg), J. Campos-Ortega (Köln), W.A. Catterall (Seattle), C. Cepko (Boston), M. Chalfie (New York), P. De Camilli (Milano), N. le Douarin (Nogent-sur-Marne), C.S. Goodman (Stanford), C. Goridis (Marseille), P. Greengard (New York), E. Herbert (Portland), E.R. Kandel (New York), R.B. Kelly (San Francisco), A. Klarsfield (Paris), D. Lancet (Rehovot), R.J. Lasek (Cleveland), M. Lazdunski (Nice), R.J. Lefkowitz (Durham), U. Littauer (Rehovot), R. Llinas (New York), J. Mallet (Gif-sur-Yvette), U.J. McMahan (Stanford), H. Möhler (Basel), D. Monhard (Basel), A.W. Mudge (London), S. Numa (Kyoto), P.H. Patterson (Pasadena), M. Raff (London), T. Reese (Woods Hole), D. Richter (Hamburg), B. Sakmann (Göttingen), M. Schachner (Heidelberg), R.H. Scheller (Stanford), U. Schwarz (Tübingen), E.M. Shooter (Stanford), J.P. Thiery (Nogent-sur-Marne), A. Ullrich (South San Francisco), M. Willard (St Louis).

The plenary sessions will cover:
Axonal transport and neurosecretion; receptors, channels and synapses; neuropeptides and biogenic amines; growth factors; lineage and differentiation; guidance and adhesion. In addition there will be poster sessions.

Application:
The deadline for applications is 31 May 1987. The total number of participants will be limited to 250. The Organizing Committee will notify those who have been accepted as soon as possible after 31 May. Participants will have the opportunity to present posters.

Applications should include a curriculum vitae and a brief description of research interests. They should be addressed to Dr. J. Tooze, Executive Secretary, EMBO, Postfach 1022.40, D-69 Heidelberg, F.R.G.

The registration fee, which includes daily transport to the EMBL from Heidelberg during the Symposium, lunches, and the Symposium reception is DM 180, for graduate students DM 90, and for participants from industry DM 360. Participants will be accommodated in EMBL guest houses. The registration fee does NOT cover the cost of accommodation. The registration fee should NOT be sent until you have been notified of your acceptance for the Symposium.

Organizing Committee:
H. Thoenen (Martinsried) — Chairman, J.-P. Changeux (Paris), P. De Camilli (Milano), T. Hökfelt (Stockholm), M. Raff (London), J. Tooze (EMBO, Heidelberg). (W3749)M

35th MEETING OF THE EUROPEAN TISSUE CULTURE SOCIETY (ETCS) AND THE 25th MEETING OF THE CELL TISSUE AND ORGAN CULTURE STUDY GROUP (CTOC)

20-24 September 1987, Athens, Greece

SCIENTIFIC PROGRAMME

Plenary lectures — Symposia — Workshops — Poster Sessions

The main topics will include:

1. Cell interaction and epithelial cell differentiation.
2. Regulation of proliferation and differentiation of hemopoietic cells by growth factors.
3. Gene transfer and biotechnology applications.
4. Markers of cell lineage and transformation.
5. Production and applications of monoclonal antibodies.
6. Bone, cartilage and muscle cell culture.
7. Hormones, neurotransmitters and signal transduction.
8. Oncogene products and their relevance to cell transformation.
9. Cell cycle regulation.
10. Oncogenes, growth factors and their receptors.
11. Development and evaluation of new drugs by tissue culture.
12. Endothelial and smooth muscle cells.
13. Cytotoxicity assay *in vitro* (CTOC).
14. Mechanism of drug resistance (CTOC).

The deadline for Abstract Receipt is 31 May, 1987.

Information can be requested from: Dr D. A. Spandidos, Chairman, Organizing Committee, 35th Meeting of ETCS, Hellenic Institute Pasteur, 127 Vas. Sofias Avenue, Athens 11521, Greece. (2041)M

INTERNATIONAL SYMPOSIUM ON:

STRUCTURE, FUNCTION, and REGULATION of MEMBRANE PROTEINS

Hotel Fürigen
Stansstad (NW), Switzerland
August 2-6, 1987

[redacted]

The general topics to be considered include synthesis and expression of transport systems, structure/function of membrane transport systems, Na cotransporters, exchangers and channels, and regulation of membrane transport systems. The format will include invited state of the art presentations in the mornings, posters and poster discussions in the late afternoons, and special keynote lectures in the evening.

For further information about the meeting and preliminary applications:

Dr Heini Murer,
Physiologisches Institut,
Universität Zurich,
Winterhurerstrasse 190,
CH-8057 Zürich,
Switzerland. (W3775)M

CONFERENCES & COURSES

SPECIAL ANNOUNCEMENT
"The Global Impact of AIDS"

International conference to be held at the Barbican Centre, London, England on March 8-10 1988. Organised on behalf of the London School of Hygiene and Tropical Medicine in association with the World Health Organisation. (2055)C

UNIVERSITY OF ABERDEEN
MSc in Clinical Pharmacology

Applications are invited for a 12 month full-time postgraduate course leading to the degree of MSc in Clinical Pharmacology. The course consists of lectures and practical work, in addition to an original research project. The course is designed for medical practitioners or non-medical graduates with appropriate qualifications in Pharmacy or Pharmacology or a related subject.

Further details may be obtained from the Secretary, Clinical Pharmacology Unit, Department of Medicine & Therapeutics, University of Aberdeen, Foreserthill, Aberdeen AB9 2ZB, to whom application for admission to the course should be made. (2085)C

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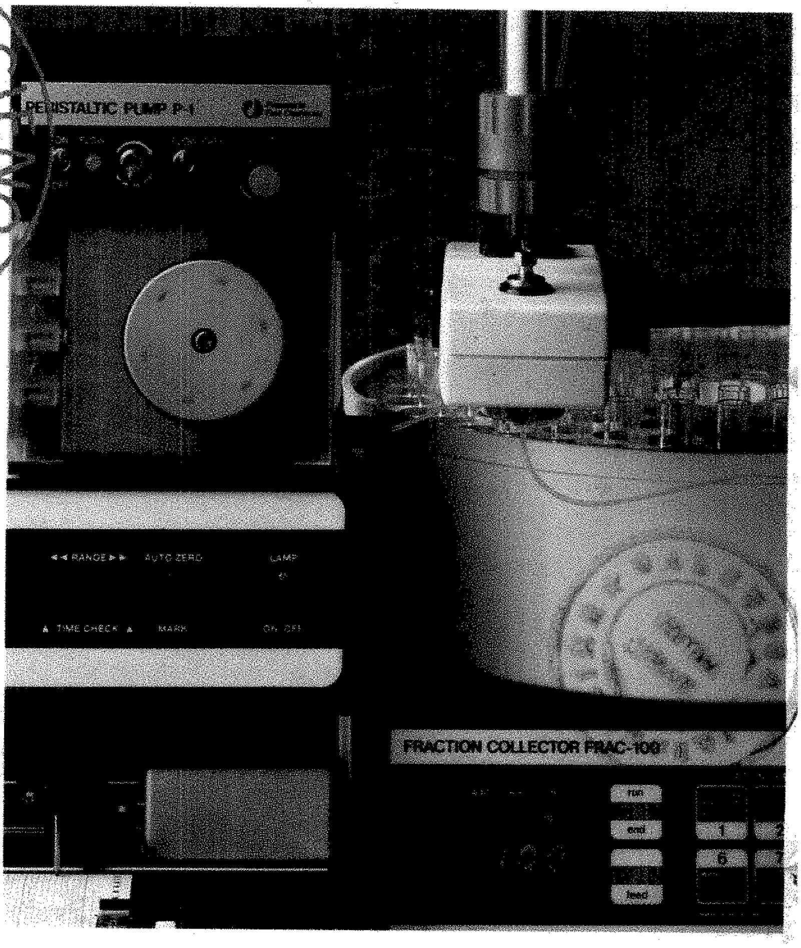

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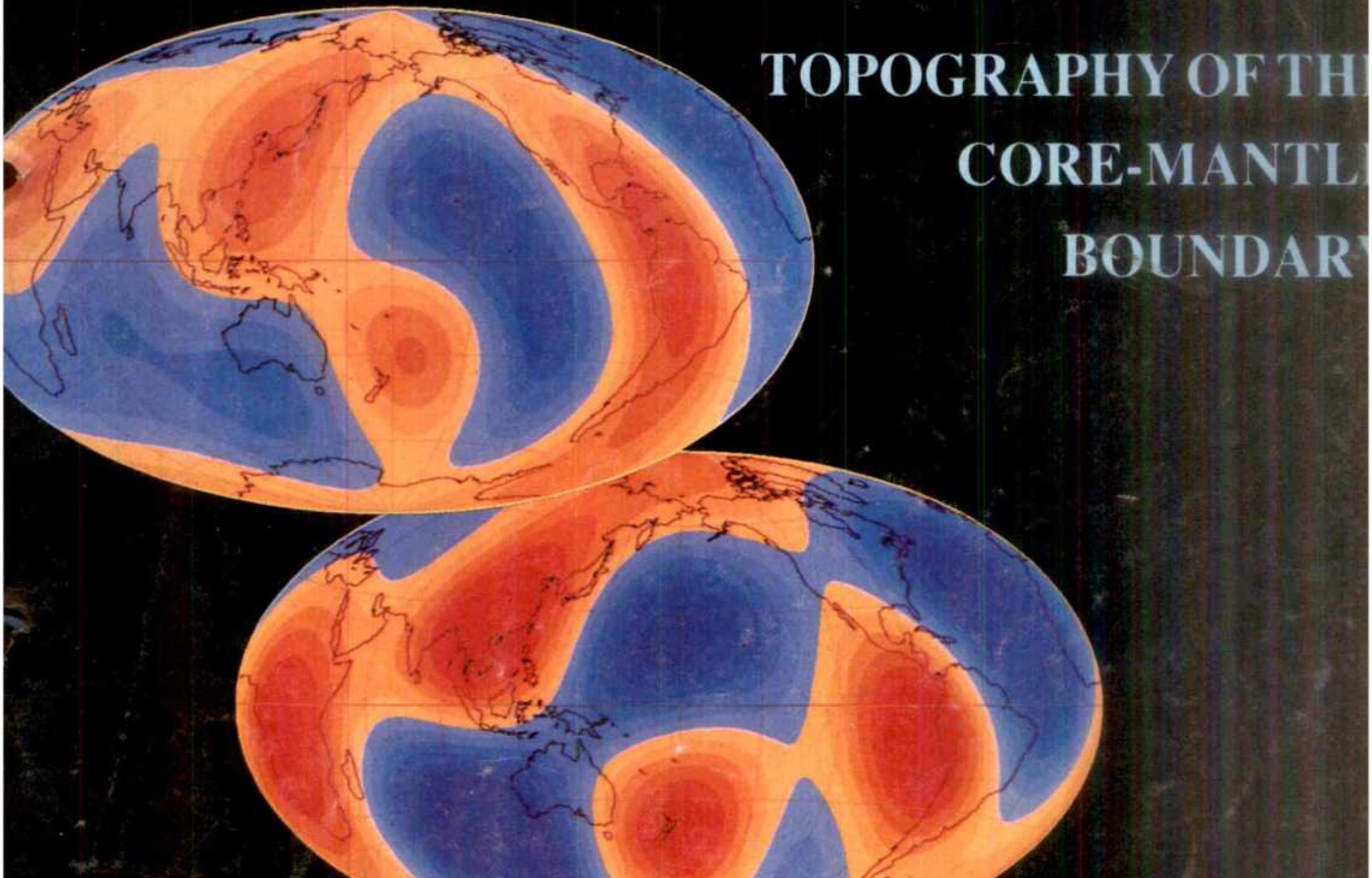

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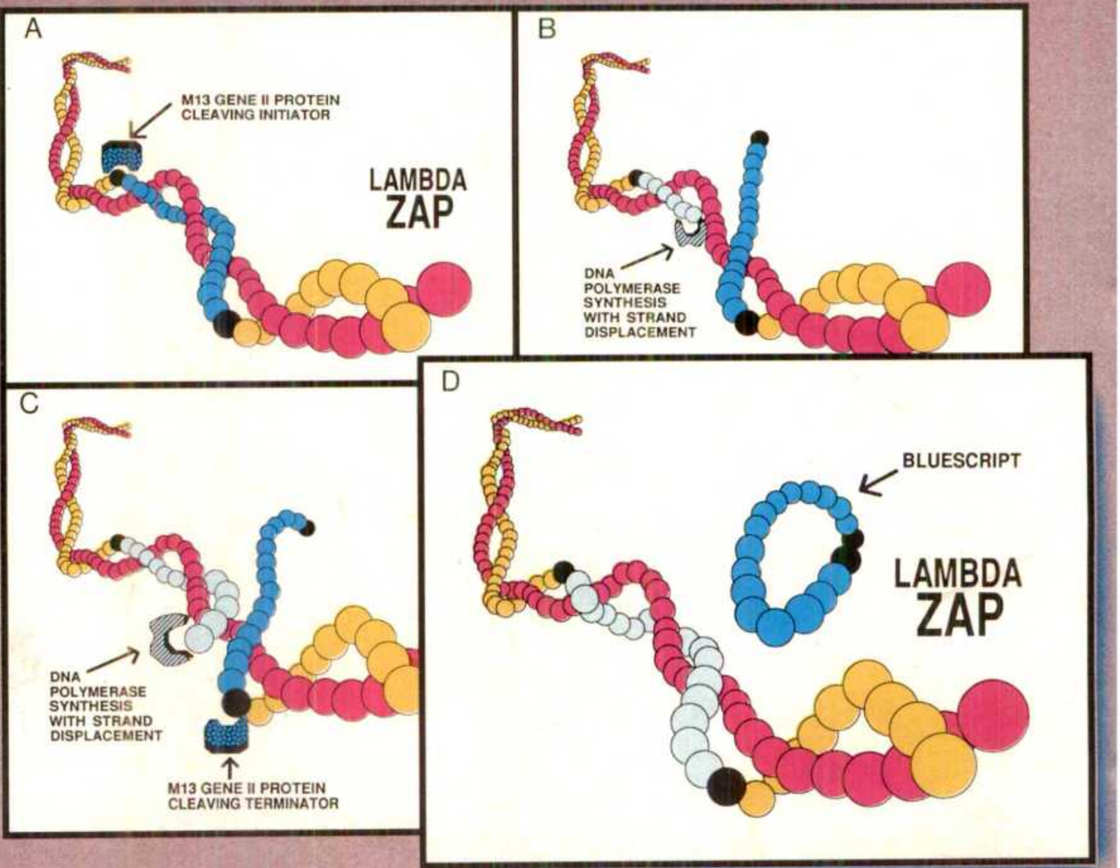


A
M13 GENE II PROTEIN CLEAVING INITIATOR
LAMBDA ZAP
B
DNA POLYMERASE SYNTHESIS WITH STRAND DISPLACEMENT
C
DNA POLYMERASE SYNTHESIS WITH STRAND DISPLACEMENT
M13 GENE II PROTEIN CLEAVING TERMINATOR
D
BLUESCRIPT
LAMBDA ZAP

Figure 1.

Automatic Excision of cDNA's from Lambda ZAP* to Bluescript

A. M13 gene II protein recognizes the signal for the start of DNA synthesis and cleaves the DNA strand at the M13 recognition site.

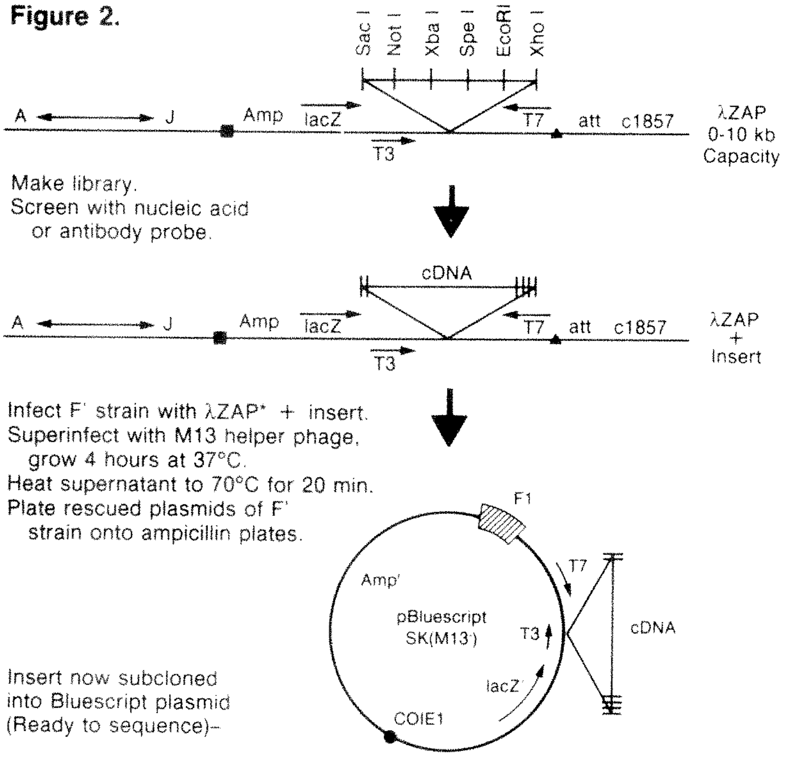
B. DNA polymerase synthesizes a new strand, displacing the existing strand.

C. M13 gene II protein recognizes the signal for termination of DNA synthesis and cleaves the DNA strand at the M13 recognition site.

D. M13 gene II protein converts the displaced strand of cDNA into a closed circular plasmid.

Breakthrough

Figure 2.



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(Ready to sequence)–

Outline of the Automatic Excision Process

The M13 helper phage supplies trans-acting proteins which recognize two separate signals positioned within the Lambda ZAP* arms. The two signals were derived from two overlapping halves of an f1 (M13) origin of replication. They are termed initiator (■), which denotes the signal for initiation of DNA synthesis, and terminator (▲) which denotes the signal for termination of DNA synthesis. Both of these signals are recognized by the M13 helper phage gene II protein. Following synthesis of a new DNA strand, the displaced strand is automatically circularized to form the Bluescript SK (M13⁻) plasmid containing the cloned insert. Since Bluescript SK (M13⁻) contains the signals for packaging as a M13 filamentous phage, the circularized plasmid is packaged and secreted from the cell. Bluescript plasmids are recovered by infecting an F' strain and plating on ampicillin plates, giving bacterial colonies

KEY

A—J : lambda structural genes
att : lambda attachment site
■ : F1 initiator
▲ : F1 terminator
MCS : Bluescript SK M13⁻ multiple cloning site, containing EcoRI, SacI, NotI, XboI, SpeI and XhoI unique sites in Lambda ZAP.
T3/T7 : RNA polymerase promoters

Stratagene's revolutionary new Lambda vector eliminates time consuming subcloning. cDNA's contained in Lambda ZAP* are automatically excised from the phage vector into the plasmid vector, Bluescript SK M13⁻ (see Figures 1 & 2) (1). The Bluescript SK M13⁻ vector (2) generated by this excision process has been specifically designed for experiments involving:

1. Exonuclease III/mung bean nested deletions
2. DNA and RNA sequencing
3. Site-directed mutagenesis
4. Synthesis of RNA probes
5. Expression of proteins
6. Isolation of single-stranded DNA
7. Production of capped RNA transcripts
8. Translation of capped transcripts.

In addition, Lambda ZAP* provides most of the advantages of other lambda expression vectors, such as Lambda gt11. The blue/white color selection on IPTG/X-GAL plates allows verification of the low background and high titers obtained from libraries made with Stratagene's pre-prepared Lambda ZAP* arms.

Lambda ZAP* provides six unique cloning sites which can hold inserts up to 10kb in length. Also, the XhoI, SpeI, and XbaI sites in Lambda ZAP* allow the cloning of MboI, BamHI, or HindIII digested DNA using the partial DNA fill-in technique of Stratagene's Lambda FIX.

(1) Short, J.M., Fernandez, J.M., Huse, W.D. & Sorge, J. (1987) Manuscript in preparation.
(2) Fernandez, J.M., Short, J.M., Renshaw, M., Huse, W.D. & Sorge, J. (1987) GENE (submitted).

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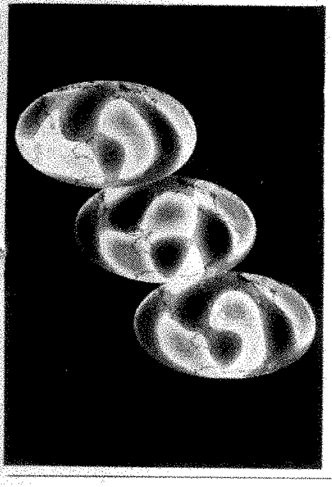


Models of undulations at the boundary between the Earth's core and mantle, from −6 km (orange) to +6 km (dark blue). Agreement between models based on rays reflected off the outer liquid core (top) and on rays passing through it (middle) support the idea of lateral homogeneity of the core. The two models are combined in the third image. See page 678.

THIS WEEK

■ Big spenders

. . . pages 651 and 654

Progress is made towards funding the sequencing of the human genome and the building of the Superconducting Super Collider in the United States and a protein engineering institute in Japan.

■ New T-cell receptor

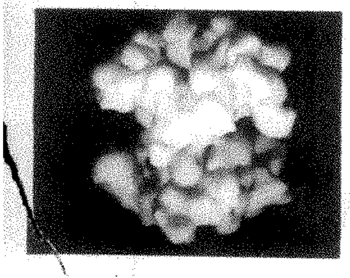
. . . pages 660, 683 and 689

A small population of T lymphocytes carry on their surface a distinct variety of the T-cell receptor protein, containing the gamma-chain.

■ Photosynthesis

. . . pages 663 and 730

Determination of the structure of a key enzyme in photosynthesis may be an important step towards improving crop productivity by protein engineering.



■ Active galaxies

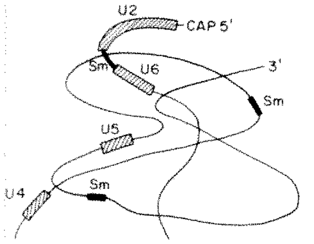
. . . pages 666, 694 and 696

Detailed analysis of the data on X-ray variability of active galactic nuclei from the EXOSAT satellite may allow some fundamental properties of these galaxies to be determined.

■ Gene splicing

. . . page 673

The role of small nuclear ribonucleoproteins in the splicing of primary RNA transcripts is reviewed.



■ Forest felling

. . . page 707

Measurements of the acid and anion content of the soil and streams after tree felling show that the forest soil has a greater than expected ability to act as a sink for sulphate ions, a major component of acid rain.

■ Alzheimer's protein

. . . pages 658 and 733

Isolation of the cDNA for the precursor of a protein fragment associated with abnormalities in the brains of patients with Alzheimer's disease and Down's syndrome has allowed the protein's gene to be localized to chromosome 21.

■ Estuarine predators

. . . page 710

The appetite of ciliated protozoa for bacteria is 10–100 times greater than earlier estimates, making them the major predators of bacteria in river estuaries and simplifying the food chain in these waters.

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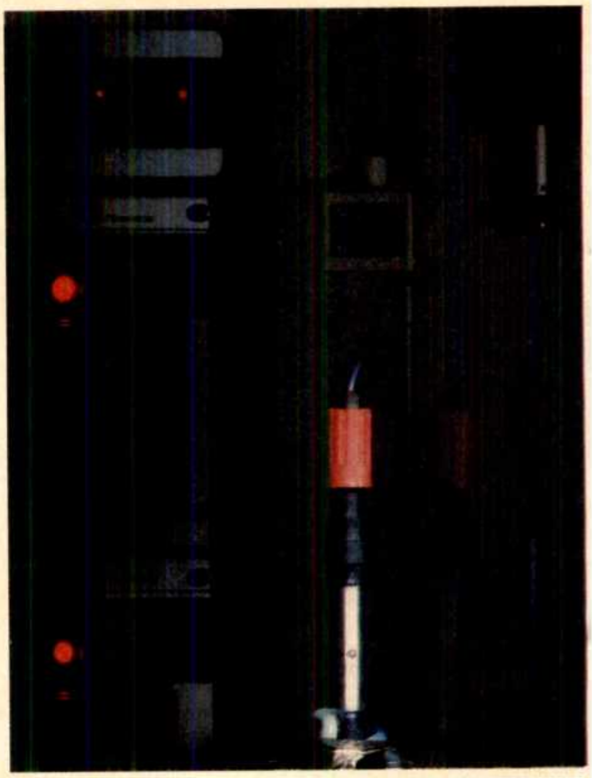

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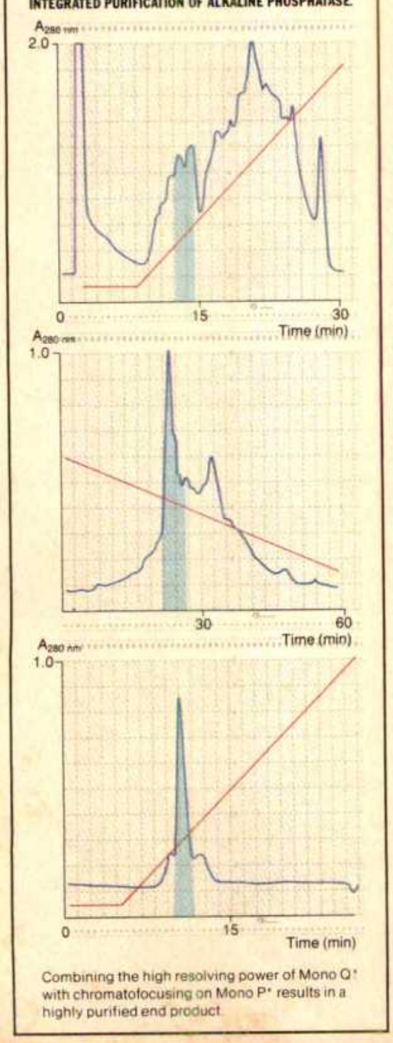

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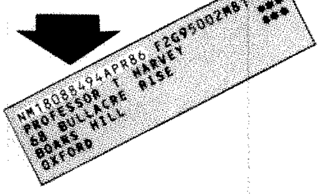

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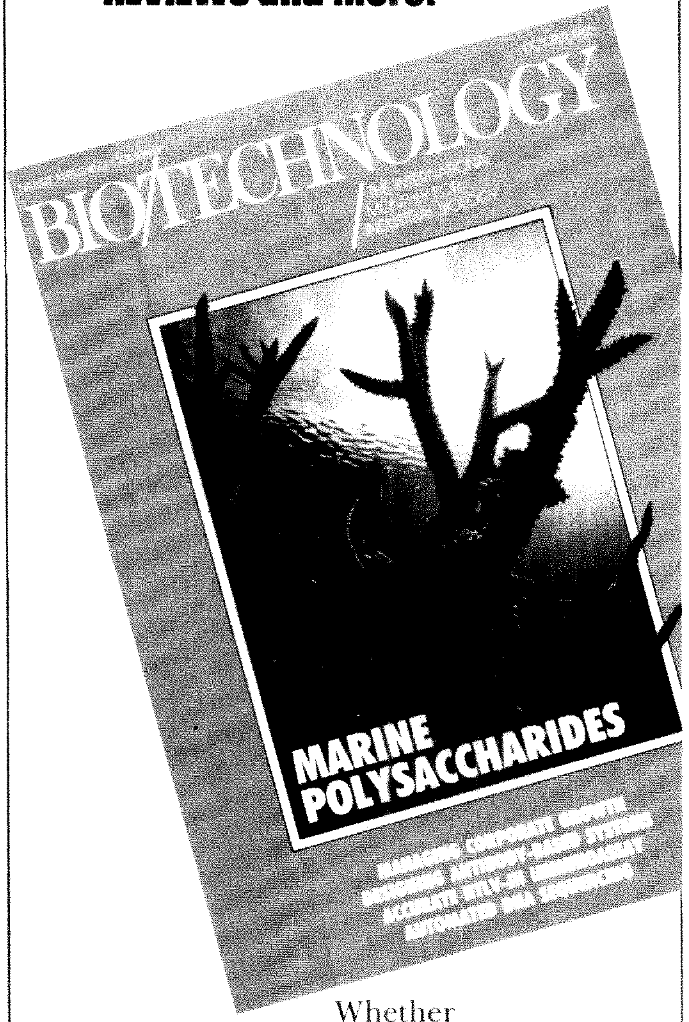

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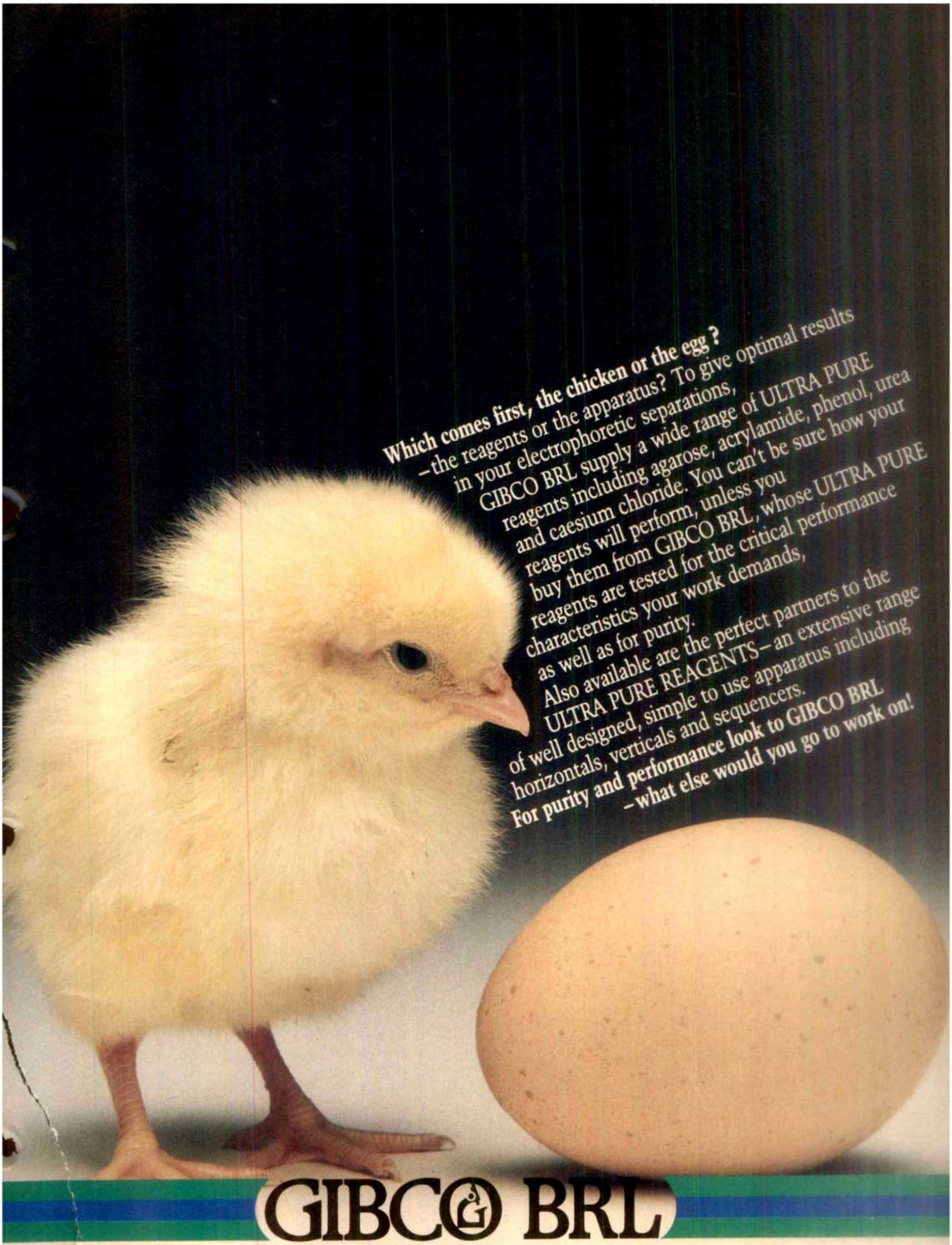

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CONFERENCE PROGRAMME

THURSDAY JULY 2

ANIMAL IMPROVEMENT

8.30 Registration

9.10 Introduction, John Maddox, Nature

9.15 Transgenic techniques
R. M. Moore/D. J. Powell
AFRC Institute of Animal Physiology, Cambridge, England

10.00 Poultry improvement
G. Bulfield
AFRC Animal Breeding Research Organisation, Edinburgh, Scotland

10.45 Coffee and exhibition viewing

11.15 Milk as a source of transgene products
J. Clark/P. Simons
AFRC Animal Breeding Research Organisation, Edinburgh, Scotland

12.00 Genetic enhancement of food and fibre value
G. Goldspink
Royal Veterinary College, London, England

12.45 Lunch and exhibition viewing

PLANT IMPROVEMENT

2.15 Nitrogen fixation
J. Postgate
Sussex University, England

3.00 Seed proteins
B. Miflin
Ciba-Geigy, Basel, Switzerland

3.45 Tea and exhibition viewing

4.15 Monocotyledons
B. Hohn
Friedrich Miescher Institute, Basel, Switzerland

5.00 Mitochondria and cytoplasmic sterility
C. Leaver
Edinburgh University, Scotland

5.45 Cocktail Reception

FRIDAY JULY 3

ANIMAL PROTECTION

9.10 Introduction

9.15 Foot and mouth disease vaccines
F. Brown
Wellcome Biotech, Pirbright, England

10.00 Rabies vaccines
J. Lécocq
Transgène, Strasbourg, France

10.45 Coffee and exhibition viewing

PLANT PROTECTION

11.15 Baculoviruses
D. Bishop
NERC Institute of Virology, Oxford, England

12.00 Frost protection
S. Lindow
University of California, Berkeley, U.S.A.

12.45 Lunch and exhibition viewing

2.15 Viral resistance
D. Baulcombe
AFRC Plant Breeding Institute, Cambridge, England

3.00 Herbicide resistance
G. Kishore
Monsanto, St. Louis, U.S.A.

3.45 Bacterial and fungal resistance
R. Dixon
Royal Holloway College, Surrey, England

4.30 Closing summary

There will be ample opportunity for discussion within the programme as each formal presentation will be followed by a question period.

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- meet international research specialists
- discuss what you learn with new contacts from all over the world

ABSTRACTS, references for further reading material and information on the speakers will be provided in advance to all participants

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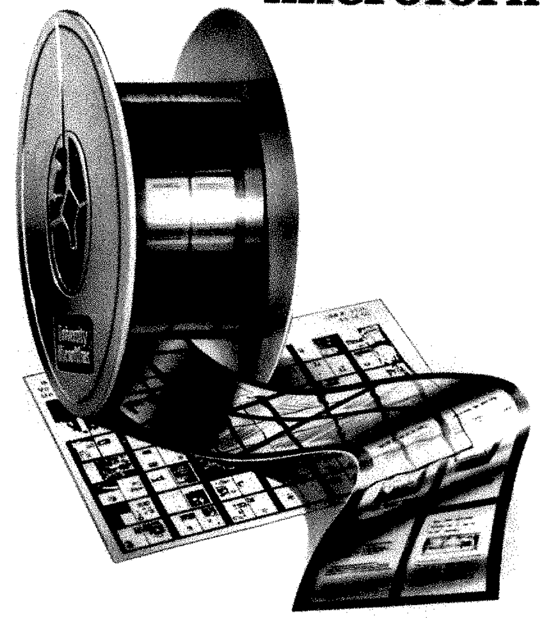

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nature

AIDS and illiberal measures

Personal liberty should be protected in the battle against AIDS but not at all costs. Registration of infected individuals makes little sense but the testing of anonymous blood samples should proceed.

It was always on the cards that panic about the spread of AIDS (acquired immune deficiency syndrome) would engender illiberal adaptations of social principles long regarded as essential safeguards of personal liberty. As long as there is neither cure nor prophylactic for the disease, and while it remains possible that all those who become infected will eventually die (it is too soon to know what the proportions are), people are right to be alarmed that whole populations are at risk. The questions that arise are those familiar during wars: what restrictions of liberty are necessary to win and at what point, along that road, does illiberality make victory hollow?

Governments' first reactions (most creditable, so far, in Britain) have rightly been that public education must be the first line of defence. The obvious difficulty is that there is no record of success in changing people's sexual habits by exhortation and every reason to fear that the social groups that are both most vulnerable and most effective as agents of the spread of infection (young people chief among them) will be immune from persuasion. Another is that the sexual explicitness essential if public education is to be effective is itself socially disturbing. Already, in the United States, the Public Health Service and the Department of Education have been at odds (*Nature* **325**, 287; 1987), while church groups in North America and Western Europe, including those that have made chastity a profession, are predictably beginning to dig in their heels. So people are beginning to cast around for other things to do.

The proposition that found its way onto last November's ballot in California (and which was thrown out) would simply have required that the condition of those known to be infected with the AIDS virus should be publicly registered as such. During the past weeks, similar ideas have been widely canvassed in West Germany (see page 650). On the face of things, they have a beguiling logic. If the threat to society is as serious as it could be, would it not make sense that those capable of transmitting the disease should in principle be identifiable and thus avoidable by those, for example, who might be about to go to bed with them? Unfortunately for the logic, there is not much chance that intending partners overtaken by sexual emergency would be diligent at consulting public registers, which would instead be used for other purposes, not all of them socially benign. The only enduring consequence of registration would be general ostracism of the infected, the principle of the old leper colonies. Would it make sense to make such a big leap backwards when other more seemly remedies are not exhausted?

There is, moreover, no good reason for believing that AIDS registration would be useful for any of the practical purposes for which it is at present canvassed. The claim that registration, even if it were only to take the form of a card carried by each individual proving that he or she had been tested for AIDS, would enable those at risk to avoid dangerous sexual partners, is largely denied by the circumstances of the case. The only tests for AIDS at present widely applicable are tests for antibody to the virus, which makes its appearance only some time after infection, perhaps a few months on the average.

None of this is an argument against testing. Especially as programmes of public education take hold in people's minds, those who think they have been running risk will look for means of telling what the truth is. They will discover that reassurance is hard to come by. Testing facilities are mostly inadequate. Moreover, as the old-fashioned venereal-disease clinics know too well, there are awesome problems of confidentiality. Obviously the person who seeks testing must be told the result, but should the testers also have powers to trace and find all sexual contacts? On the face of things, the universal adoption of such a policy would make good sense. But there are horrendous difficulties. First, there is the obvious problem that those at risk are less likely to offer themselves for testing if they know their contacts will be traced. Then there is the difficulty of conducting an investigation of a person's life without casting a slur over all those concerned. There is also the problem that the tracing and testing of all sexual contacts of an infected person is expensive both of people's time and of money, especially if it is accompanied by proper attention to the counselling of those concerned. At some stage in this battle, it may seem that the compulsory tracing of the sexual contacts of infected people would be worthwhile; but nobody should expect too much of it. Meanwhile, a more subtle moral issue has arisen, both in Britain and the United States (where public health authorities have been beating their breasts on the subject for the past few weeks). The question is whether it can ever be proper to attempt to estimate the incidence of infection in the population at large, a number crucial to serious attempts to chart the future of the disease, by the anonymous testing of blood taken from patients for quite different purposes. A group of physicians in Britain has been asking for months for this course to be followed with blood sampled during pregnancies.

Anonymous testing would at present be disallowed because it violates the cornerstone of codes of ethics which lay down that people should be asked to give their consent to what is intended. Another ethical objection to the procedure is that the anonymous people who were found to be infected could not be given this information, even if they would find it valuable. These are issues on which governments and professional bodies should be prepared to bend the present rules. Nobody whose blood was tested anonymously would be the worse off, whatever the result of the test. Moreover, the data that could come from testing of this kind would be an invaluable means of monitoring the progress of the disease. If the battle against AIDS were a real war, there would be no doubt of what should be done. ☐

Where to go next?

The House of Lords has told more truth than most on UK science: now it has another chance.

Is the quality of research too important to be left to politicians and civil servants? And is the dreadful condition into which the British research enterprise has been driven in the past seven years the result of the way in which government departments have been free to do what seems to them to be sensible, unhindered by protests from people in a position to know what damage their decisions would cause? These are the sceptical questions which have been implicit in the modest stream of constructive criticism which has come, during this unhappy period, from the only consistent source of commentary on the condition of the British research enterprise — the House of Lords Select Committee on Science and Technology. From the outset, in a report published in 1981, the committee has been pleading for a mechanism by which science and technology, in their own right and not as ingredients of some department's larger programme, should have some kind of political representation in the machinery of government. Last year (see *Nature* **325**, 95; 1987) the committee repeated its demand in an even more persuasive form: not merely should there be a minister (other than the Prime Minister, who has a science degree) but also an advisory council including independent members that would have a say in the direction of events. The members of the committee, and the House of Lords in general, will have a chance further to pursue this cause in the debate arranged for Thursday of this week. They should push hard, but prudently (which is their habit).

Much of what has damaged British science in the past fifteen years or so has been done in the name of good sense. In the old days, two decades ago, there were no difficulties in the allocation of resources (for technical people knew what the needs were), but even the governments of those halycon days (and their successors) would have benefitted from a means of knowing whether the funds spent so generously were also spent wisely and effectively. When it first became apparent (in the early 1970s) that it would not be possible for budgets to keep on rising, but that they might even have to shrink, those concerned did not understand the system well enough to know what should be done. It seemed sensible when those research councils whose prime function is the support of basic science responded to the prospect of stagnation by creating common facilities in principle accessible to all academic scientists; these same institutions, fifteen years later, are a large part of the reason why the support of creative people has become inflexible. Similarly, with the recent need, in Britain's serious economic condition, to cut back on public spending, it made sense for research to be put on the auction block. Ironically, the government's proper wish that, in the interests of good management, government departments should be responsible for their own spending meant that decisions about what to cut were left to people even less able to understand the difficulties. The way that the Ministry of Agriculture, Fisheries and Food has, during this period, played ducks and drakes with the research council responsible for agriculture is nothing but a scandal.

The lessons to be learned from this unhappy period are that politicians and civil servants have jointly ruined a previously splendid enterprise for the lack of a framework of policy within which to carry out their well-intentioned but often mistaken decisions. So much can be told from the way that government departments have recurrently been swept by fashions — such as the fad for information technology and 'relevance' — and have sought to attain worthy objectives by methods fated to be ineffectual, attempting to skew the pattern of research while neglecting the need for mechanisms to ensure that useful outcomes can be successfully applied. At the same time, important ingredients of the equation "research success = prosperity" have been left untouched — British military research remains too far apart from the rest of the British enterprise. During this whole period, these errors of commission and omission would have been less easily foisted on the system if there had been a formal (if small) part of the machinery of government with the authority to question political decisions touching science and its application. For the advice to be that of an independent council would have the further benefit of helping to bring important issues out into the open and of providing a measure of continuity conspicuous by its absence in the management of British research. The House of Lords report had much else to say last year, but this is the nub of its demand. Can the government say no yet again? ☐

Shades of green and blue

The hostile reception to UK government plans to promote other uses of farmland is undeserved.

With one wave of a £25 million wand, Britain's Minister of Agriculture, Mr Michael Jopling last week tried to turn beef into buildings and butter into beech woods. So began what is sure to become a growing campaign on the ground in Britain, as opposed to in the corridors of Brussels, to shrink the food mountains of Europe. An edifice to the sluggishness of Euopean politics, these mountains have continued to grow at the expense of the taxpayers who heavily subsidize farmers to produce more food than Europe can consume. Since there cannot be a British taxpayer who whole-heartedly approves of this, unless he is also a farmer, Mr Jopling (who happens to be a farmer) must have hoped for a generally favourable response to his plans to entice farmers, by means of money, to find alternative uses for some of their less-good land. Why, instead, was his wand-waving greeted with the kind of reception that is usually reserved for the wicked fairy in the Christmas pantomime?

One justifiable reason would have been that the alternatives were too limited. But they are not. Of the £25 million, £10 million is to be offered to persuade farmers to turn grassland into woodland, £7 million to pay them not to harm so-called Environmentally Sensitive Areas, and smaller sums to encourage forestry as well as farmhouse industries such as cheese-making and providing clotted cream teas for tourists. Moreover, except for the very best of land, the constraints on converting it to non-agricultural use are to be relaxed — that is, if the local planning authorities see fit to allow a mediocre field to be turned into a golf course or a cluster of 'exclusive georgian-style residence', they need no longer take into account the loss of potential beef, butter or milk.

Farmers, however, can small a rat a mile off. While having no objection to the prospect of selling at great profit a field or two to a property developer, they know that in general the changes are a harbinger of much worse to come from a government set on reducing public expenditure in general and farming subsidies in particular. Nor were those whose prime concern is for the environment to be found rooting for the minister. This being an election year, Mr Jopling had clearly hoped to gain 'green votes' by some of his measures. For example, by doubling the budget available for paying farmers to stick to traditional methods in Environmentally Sensitive Areas, it will be possible to ensure the protection of some more areas from the waiting list. And one cry of all environmentalists has been at least partly heeded by the promise that a certain proportion, perhaps 20 per cent, of any woodland planted under the proposed scheme of additional subsidy will have to consist of broad-leaved trees. Where Mr Jopling lost the 'green vote' — and is in danger also of losing the 'blue vote' of traditional Conservative voters from commuter belts — is in relaxing the restrictions on non-agricultural use of farmland, particularly with the ensuing prospect of urban sprawl.

It is possible to feel some sympathy for Mr Jopling. After being careful to feed the barracudas before dipping his toe in the water, he still found them hungry enough to attack. He and his counterpart in the Department of the Environment are now going to have to consider how best to proceed. The Environment Secretary would be wise to say more on how planning authorities are to interpret the relaxation of the agricultural factor in considering applications for redevelopment of farming land. After all, he is the final court of appeal for developers denied permission. But that apart, the best course of action of the government is to stand firm and carefully monitor the results. For this is only the start of a programme that will, by some accounts, convert 30 per cent of English farmland to other uses by the end of this century. Viewed in that light, Mr Jopling's pilot experiment is likely to do more good than harm. ☐

World eavesdrops on the Moscow 'peace' conference

- Sakharov challenges both superpowers
- Gorbachev sounds a cautionary note

London

LAST weekend, an International Forum "for a non-nuclear world and the survival of humanity" took place in Moscow. Formally the 1,000 plus participants came as 'individuals', and the meeting had no connection with any government, including that of the Soviet Union. This did not inhibit Mr Mikhail Gorbachev from entertaining the delegates to a closing reception at the Kremlin, where he delivered a major speech, castigating the United States for "effectively trying to scrap the Anti-Ballistic Missile treaty of 1972" and warning that the Soviet Union would be resuming the testing of its own nuclear weapons. But the concept of individual participation did allow the organizers to invite Dr Andrei Sakharov to participate, thus attracting the attention of the world media.

Sakharov's presence was an entirely voluntary act. His personal campaign for nuclear disarmament goes back almost twenty years — indeed, he made his debut as a dissident with an essay on "progress, peaceful coexistence and intellectual freedom" which was triggered by his concern over the arms race. Moreover, his stance on the US Strategic Defense Initiative (SDI) was formulated in an article in *Foreign Affairs* in 1983: namely, that for such a system to operate as an adequate deterrent, it would have to achieve a degree of precision not feasible within the current or foreseeable state of the art. This same argument was advanced earlier this month by a spokesman for the Soviet government, Gennadii Gerasimov, who compared the degree of precision involved with that needed to produce a library of several thousand volumes without a single misprint. At this point Dr Sakharov's views coincide with those of the government. This may well have influenced those who decided to end his exile — although they do not challenge his credibility of independence of thought.

The only limit, as far as this particular forum was concerned, was the external one imposed by the Soviet media. For, in his address to the working group of scientists he criticized both superpowers, saying on the one hand that "the West must not try to corner the Soviet Union", for "a cornered nation is always dangerous", but on the other hand that a "more democratic and open society" in the Soviet Union (including the right to emigrate from it) would be a major safeguard of peace. The Soviet media, however, reported only his criticisms of the Western stance and of SDI technology, ignoring his remarks abut the Soviet Union.

Dr Sakharov's presence, while focusing world attention on the forum as a whole, nevertheless meant that some other issues were overlooked in official statements. (The sessions themselves were closed.) Nothing was publicly said, apparently, of the missing computer scientist Vladimir Aleksandrov, who worked on the Soviet computer model of a possible 'nuclear winter' and who vanished from a peace conference in Madrid in spring 1985, although his continuing absence is itself a source of international tension among his former colleagues. Nor was much notice paid by the West to the peace initiatives of the Bulgarian-launched 'Ecological Forum', several of whose leading members were present in Moscow. One of the movement's founders, Dr Nansen Bekhar, explained during a visit to Britain last month, that this body aims to be not simply a "professionals for peace" group, but to work actively for the elimination of environmental problems which can themselves provoke or exacerbate international tensions.

Yelena Bonner and Andrei Sakharov in their Moscow home.

As far as the Moscow meeting was concerned, Dr Sakharov's rehabilitation seems to have been complete. He was present at the Kremlin reception where he warmly applauded Mr Gorbachev's statement of his commitment to "new approaches on humanitarian issues". But to those conference participants who attended the forum in the hopes of influencing Soviet policy, Mr Gorbachev sounded a warning — the new response to humanitarian issues was not, he said, a response to pressure from the West. This stance tallies with the statement by Central Committee member Georgii Arbatov last weekend that the release of the Jewish activist Iosif Begun was actually delayed by the Moscow protests on his behalf. The Soviet leaders, it would seem, are prepared to exercise a new clemency, but do not wish to be seen as acting under compulsion.

Vera Rich

Space station partnership still in jeopardy

Washington

INTERNATIONAL collaboration in the US space station remains in the balance, but less precariously than before last week's meeting of delegations from the US and potential partner governments. The statement issued after the meeting says that Canada, Japan and the members of the European Space Agency (ESA) will continue their negotiations with the United States towards a civil space station. But the National Aeronautics and Space Administration (NASA), the prime mover of the project, seems as worried by internal as by international threats to the project.

The objective of allowing the three non-American partners to satisfy themselves that the space station will not be dominated by the US military seems only partly to have been attained. The statement saying that negotiations are to continue gives no timetable, even though ESA, for one, needs to know where it stands by its next council meeting in June.

Observers who accept NASA's contention that it has never hidden the possibility of US military participation admit that some partners may have been shaken by the prospect of the space station being used for testing components of the Strategic Defense Initiative. The need now is for a form of words assuring non-military users that their projects will not be overridden by military needs while not usurping NASA's overall responsibility for managing the station. Part of the problem is that there are two agreements to be negotiated, one among the participating governments and one between NASA and other operating agencies such as ESA.

Both the programme office and NASA's comptroller have carried out reviews of the project, which have in turn been reviewed by Dr James Fletcher, NASA's administrator. Fletcher is now negotiating with the White House's Office of Management and Budget about the cost, believed to be much greater than the $8,000 million originally forecast. Whatever the outcome, some delay seems unavoidable. By 1994, the date first fixed for full operation of the space station, the best that can be hoped for is the launching of some parts of it.

Terence Finn, a deputy director at NASA's space station office, says that the station could allow the United States to regain its leadership in space science. Accommodating the Pentagon's interests should be feasible but will require diplomacy, he says. He admits to worry that the Department of Defense might become "insensitive to the international and research flavor" of the space station. ☐

AIDS registration becoming a political issue in Germany

Hamburg

THE control of AIDS (acquired immune deficiency syndrome) is gaining political momentum daily and is now generating as much public anxiety as unemployment, foreign affairs, environment and tax. In the wake of last month's general election, the winners — the Christian Democrats (CDU), their sister party CSU and the Liberals (FDP) — have been negotiating to install a new Christian-Liberal coalition and have witnessed AIDS gaining political stature. Compulsory registration of AIDS victims is now a prime issue.

Franz-Josef Strauss, Minister President of the state of Bavaria and head of the CSU, has asked his partners to discuss the epidemiological aspect of the disease. He and his party demand a compulsory registration for all who are infected with human immunodeficiency viruses — similar to the strategy adopted in Sweden.

Newspapers daily report the disease in big headlines, mentioning new cases and speculating on the chances of infection. AIDS had been ignored for a long time by West German politicians. The Health Committee of the Bundestag (parliament) was briefed for the first time in autumn 1985. One year on, the first plenary discussion took place; it lasted only half an hour. The result was a commitment to research and public information.

The Minister of Health, Heiner Geissler, did almost nothing about AIDS during his period in the Cabinet. His successor, the Christian Democrat Rita Süssmuth, who came into office in September 1985, did not share Geissler's opinion that AIDS was a problem only of the high-risk groups. Her ministry started a campaign of enlightment, paying millions of marks for advertising in newspapers and magazines, television, folders and posters. A current youth centre poster declares "Carnival will be over on Ash Wednesday, AIDS won't".

DER SPIEGEL
SCHUTZ VOR DER SEUCHE:
Meldepflicht für AIDS?
SPIEGEL-Gespräch mit Bundes-Gesundheitsministerin Rita Süssmuth

Süssmuth is an enthusiastic proponent of the use of condoms — she was photographed for the magazine *Der Spiegel* in a huge plastic bag. Her campaign is supported by the Liberals, Social Democrats and Greens. She rejects, however, compulsory registration, at least at the moment. Her political influence will very much depend on the success of her campaign. In Berlin the campaign has considerably reduced the number of cases of venereal disease. At the end of January there were 875 individuals in West Germany registered with AIDS, out of whom 409 have already died. Süssmuth estimates that the number of infected persons lies between 30,000 and 100,000 and that by 1990 half a million West Germans will have the virus.

CSU health expert Kurt Faltlhauser wrote a paper for the CSU members of parliament in Bonn in which he demanded compulsory AIDS tests for risk groups such as haemophiliacs, drug addicts and registered prostitutes, as well as for all pregnant women and for travellers and foreigners from "risk countries". A voluntary test (anonymous and free of costs) is also to be offered to every resident of West Germany.

At a later stage, the CSU expert recommends "compulsory tests and registration for the whole population", for which the preparations must be made now. Friedrich Zimmermann, CSU Minister of the Interior, even considers restrictions on infected persons. CSU MP Erich Riodl demands the separation of groups in places where people have to live together such as schools, clubs and factories.

The Liberals also demand an 'AIDS ministry'. Part of the Süssmuth Ministry and parts of the Ministry of Labour should be put together to create a new Health Ministry, which could better cope with the growing problem. **Jürgen Neffe**

MRC pushing for government funds for AIDS vaccine research

London

WITH an annual budget that would soon be £10 million, it would be possible to organize and direct an effective AIDS (acquired immune deficiency syndrome) vaccine research programme in the United Kingdom.

The claim is that of the Medical Research Council (MRC) which has devised the blueprint of such a plan, currently being studied by government. The disclosure was made last week to the House of Commons Social Services Committee as it took evidence from the MRC on AIDS.

The plan, submitted to government through Mr Kenneth Baker, Minister for Education and Science, at the end of December, outlines a strategy that will give the council extra AIDS funding of £2.5 million in the first year, rising to £4–6 million in the second and then £10 million before the fifth year.

In the written evidence to the committee the MRC had claimed: "We believe that the UK has the scientific capability for making a decisive contribution to the development of an AIDS vaccine provided that a political lead is given and that substantial extra funding, outside the Science Vote, is forthcoming. A number of distinguished scientists in the UK have expressed a wish to take part in a programme of research to develop an AIDS vaccine."

The paper, supplemented by oral evidence principally from Lord Jellicoe and Sir James Gowans, paints a grim picture. To determine the behaviour of the disease, groups of infected individuals need to be studied for another 20 years or more, concludes the MRC. But a new British programme to find a vaccine should be mounted and the MRC would speedily allocate the funds through its mechanisms for 'special project grants'.

The MRC, which created a working party on AIDS about 4 years ago, has since provided eight special grants for AIDS research from its own funds and has diverted resources at its own establishment, the Clinical Research Centre at a total cost of £700,000 a year. Also seven 'special allocations' have been made for epidemiological studies, funded by the health departments to the tune of £300,000 a year.

The council was recently awarded an extra £1 million a year to pay for further AIDS research. Additional research in Africa, where the MRC already supports projects in The Gambia and Zambia, is now also expected to be financed.

The MRC evidence emphasized that general screening of the population for human immunodeficiency virus infection raises difficult ethical and legal questions. But it concludes that: "There is a need now both for extensive facilities for the voluntary testing of all sexually active members of the population who are willing to know if they are infected, and the testing of representative samples of the population to monitor the spread of infection". **Bill Johnstone**

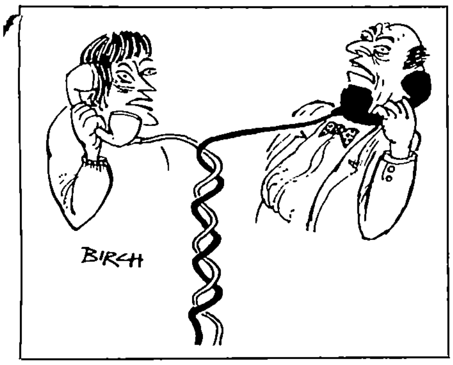
Protein engineers to gain from telecommunications selloff

Tokyo

UNEXPECTEDLY, the privatization of Nippon Telegraph and Telephone (NTT) appears to have benefited Japan's molecular biologists. A small part of the massive funds flowing into the Japanese government coffers from the privatization will be channelled into the creation of a protein engineering research institute.

Last week NTT emerged on the Tokyo stock exchange as the largest private company in the world. Although sceptics considered the stock overvalued at ¥1.2 million ($8,000) per share, trading in NTT shares was paralysed for two days as buyers outnumbered sellers by a hundred to one. Only when the government released a further 100,000 shares on the market did trading get under way at ¥1.6 million, and by the weekend the price had risen to nearly ¥1.8 million. If the price holds, the government stands to make over ¥13 million million from the sale of 50 per cent of NTT during the next three fiscal years.

While most of the money accruing from the privatization is expected to be swallowed by the government's huge fiscal deficit (debt servicing payments currently run at about ¥11 million million a year), a small portion is being directed into support for the Japan Key Technology Center, an organization for the promotion of basic research that was established jointly by the Ministry of International Trade and Industry (MITI) and the Ministry of Post and Telecommunications (MPT) in October 1985 (*Nature* 317, 99; 1985).



Ever since Japan Key Tech came into existence, the two ministries have been vying for the most prestigious project to be funded by it. This month MPT will establish an institute for the development of next-generation space communications, such as large-diameter antennas and miniaturized transmitters for space stations, with funding of ¥6,000 million over eight years. But this pales in comparison with MITI's plans to build a protein engineering research institute.

About two years ago MITI counselled a group of advisors about suitable projects to establish in the field of molecular biology and biotechnology. The protein engineering institute was selected from a list of about twenty ideas drawn up by the group. Established as a joint-stock corporation in April last year, the institute will receive funds of around ¥17,000 million over the next ten years. Fourteen biotechnology companies, including the 'big five' (Kyowa Hakko Kogyo Co., Takeda Chemical Industries Ltd, Toray Industries Inc., Mitsubishi Chemical Industries and Ajinomoto Co.), will contribute 30 per cent of the funds, the rest coming from Japan Key Tech.

The institute, which has temporarily set up headquarters in Tokyo, will move next spring to the former site of EXPO 70 in Suita City near Osaka, an area already endowed with several biotechnology-related institutes. The land for a three-storey laboratory is being provided rent free by the local Osaka government, which plans to build a bioscience institute next door, and it is expected that the Osaka government will take over the protein engineering institute when funding runs out in ten years.

Professor Morio Ikehara, managing director of the institute, plans to install the latest high-tech equipment, including a supercomputer and the world's most powerful nuclear magnetic resonance spectrometer (600 MHz). There will be five departments — structural analysis of proteins, correlation between structure and function, production (genetic engineering), purification plus evaluation of functions, and a database and computer service department. But projects will be drawn from every department to encourage interdisciplinary research.

But at present a battle is raging between those on the management side of industry and the academics over organization and staffing of the institute. The academics are seeking talent with ideas, whereas those in industry want good management and a quick return on investment.

David Swinbanks

Interest in the human genome project reaches new heights

Washington

THE effort to map and ultimately to sequence the human genome has captured the attention of the White House. The cabinet level Domestic Policy Council (DPC) has asked its working group on biotechnology to prepare a report on the human genome project. A first instalment of the report, due next month, will detail present US activities. Future instalments could include a framework for coordinating federal activities on the project.

The working group will meet on 23 February to decide how best to proceed. According to David Kingsbury, its chairman, the report will probably be written by a subgroup on which the National Institutes of Health (NIH), the departments of Energy and State and the White House Office of Science and Technology Policy are expected to be represented.

The first report will be primarily to educate DPC members on the costs and benefits of pursuing the genome project. The notion that such an endeavour could cost $3,000 million attracted White House attention. No federal agency is contemplating such a large project, but several are already working on parts of a sequencing and mapping project.

The plans of the Department of Energy (DoE) for the human genome project are the best defined. It is supporting the development of a set of DNA libraries specific to individual chromosomes. Work is under way at Los Alamos National Laboratory on chromosome 16 and at Lawrence Livermore National Laboratory on chromosome 19. A consortium headed by Cassandra Smith at Columbia University is working on chromosomes 21 and X.

Charles DeLisi, who heads DoE's efforts, says that the department will spend approximately $5 million on the human genome project in this fiscal year and $11.5 million next year. As well as the DNA libraries, DoE will focus on new sequencing technology and computational activities. DeLisi says that techniques for calculating structure from sequences will become increasingly important. An advisory group met earlier this month to specify plans for this year's DoE activities.

NIH, which supports several projects that will be relevant to any mapping and sequencing effort, has not yet decided exactly what its role in the human genome project will be. NIH director James Wyngaarden chairs a small working group that will guide NIH directions on the project. George Palade of Yale University serves as the group's external advisor.

Other reports on the human genome project are in the offing. The National Academy of Sciences Board on Basic Biology plans to complete an evaluation of the project by June. For Congress, the Office of Technology Assessment is also evaluating the genome project. A draft of that report may be available this summer, although it is not officially due until the end of the year.

Joseph Palca

Investigations into NIH fraud allegations end with suicide

Washington

An investigation into possible scientific fraud at the National Institutes of Health (NIH) has had tragic consequences. Apparently as a result of emotional pressure caused by an inquiry that focused on a researcher's conduct in his former laboratory, Howard Eisen committed suicide on 7 February, 10 days after appearing before a panel looking into the affair.

Eisen was in the Laboratory of Developmental Pharmacology at the National Institute of Child Health and Human Development (NICHD), and chief of the section on regulation of gene expression. The NIH inquiry focuses on a paper entitled "Analysis of mammalian P450 catalytic activities by genetic engineering and expression in yeast." According to Eisen's widow Laura, a research associate in chemistry at the Uniformed Services University of the Health Sciences, most of the data for the paper were gathered by Alok Bandyopadhyay. Laura Eisen says Bandyopadhyay was hired last summer by Daniel Nebert — head of Howard Eisen's section— to work in Eisen's laboratory. According to Laura Eisen, Nebert wrote the paper, although Bandyopadhyay was first author. Howard Eisen reviewed the paper, and he was one of the authors, she says.

The paper was intended for publication in the Proceedings of the National Academy of Sciences, but certain inconsistencies in the data were detected before it was actually submitted for publication. Those discrepancies prompted a careful review of Bandyopadhyay's work at NIH. Laura Eisen says her husband performed experiments that convinced him that Bandyopadhyay had adulterated his data. She says not only was there evidence that there were serious problems with Bandyopadhyay's research, but close scrutiny subsequently turned up numerous inconsistencies in his curriculum vitae. Unfortunately, Bandyopadhyay could not be reached for comment.

Results of this preliminary scrutiny prompted the NICHD to terminate Bandyopadhyay's employment last November. NICHD director Duane Alexander says that the NIH follows specific procedures in any case of suspected fraud, both within and without NIH. So on 8 January this year deputy director Joseph E. Rall named a three member pane with the object of looking into "serious discrepancies in the paper".

The panel comprises Maxine Singer of the National Cancer Institute and two other members, one from NIH and one from outside the institutes who acts as chair. The panel held its first meeting on 28 January, when both Howard Eisen and Bandyopadhyay were expected to be interviewed. But Bandyopadhyay never arrived, and after waiting several hours the meeting broke up with no substantive ground being covered.

Howard Eisen was extremely upset by his meeting with the panel according to his widow. She says he insisted on going back to his laboratory to make certain that his checks of Bandyopadhyay's work had been accurately prepared. Laura Eisen says her husband was not thinking rationally following the meeting. Upset from the start by the apparent fraud, Laura Eisen says "something snapped" after the 28 January meeting.

Friends and associates of Howard Eisen say the idea that he could have been involved in anything fraudulent is unthinkable. Jeffrey Harmon, a colleague of Laura Eisen at the Uniformed Services University of the Health Sciences, who had collaborated with Howard Eisen in the past, says "there's no way Howie could have been involved in any kind of cheating".

Although he had minimal contact with Eisen, Duane Alexander describes him as "as excellent scientist". NIH has made no allegations against Eisen.

Howard Eisen came to NIH in 1971. He was graduated from Harvard College in 1964 and Harvard Medical School in 1969. Eisen did a two-year residence at Boston City Hospital before joining NIH. His work has been primarily on glucocorticoid receptors.

NIH is still planning to continue its investigation into the fraud allegations.

Joseph Palca

Two British scientists share Wolf Prize

Rehovot

Two noted British biochemists will share the $100,000 Wolf Prize in Chemistry for 1987. They are Sir David Phillips of the University of Oxford and Professor David M. Blow of Imperial College, London, who are being honoured "for their contributions to protein X-ray crystallography and to the elucidation of structures of enzymes and their mechanism of action".

Phillips and Blow will receive the Wolf Prize from the president of Israel, Dr Chaim Herzog, in ceremonies at the Knesset in Jerusalem on 31 May, when awards will also be presented for achievements in chemistry, mathematics, medicine, agriculture, physics and the arts.

Sir David Phillips (left) and David Blow

Nechemia Meyers

AAAS reviewing the situation

Chicago

The American Association for the Advancement of Science (AAAS) boasted of a remarkable coup at its annual meeting here this week — the appointment of Dr Alan Trivelpiece as its chief executive officer to succeed Mr William Carey, who is retiring. Dr Trivelpiece has been for several years responsible at the Department of Energy for, among other things, US policy on high-energy physics. He is believed to be the chief architect of the US government's decision, announced two weeks ago, to back the project to build the Superconducting Super Collider (see page 654).

In contrast with previous years, the AAAS this year has no fierce complaint to make of the federal budget for the year ahead. Neither the present nor the putative executive officer could do other than applaud the hope that Congress will allow the doubling of the budget of the National Science Foundation (NSF) over the next five years, one of the outstanding ingredients of the administration's budget for the financial year beginning next October. But Mr Gerard Piel, chairman of the AAAS board of directors, "deplored" the NSF's recent practice of setting up university-based research centres whose effect, he said, is to undermine the autonomy of the host universities.

The AAAS itself seems also to be going through a period of heart-searching about its future. A downbeat statement in the association's house journal, *Science*, which records the circumstances leading to the demise of its monthly offshoot *Science 86*, also says that the future of the annual meeting is being re-assessed because of poor attendences. Unsystematic sampling of this week's sessions reveals that distinguished speakers are often as numerous as those who have come to listen. But hotels have apparently been booked for the next two years.

John Maddox

Call for replacement for UGC and bigger role for industry

London

BRITISH industrialists and women must play a greater part in influencing the education and research needs of the nation while government, via a special commission, should have immediately available expert counsel on education.

These recommendations have emerged from a committee created to review the University Grants Committee (UGC) — the principal financial watchdog of higher education. The committee, chaired by Lord Croham, chairman of the bankers Guinness Peat and former head of the Home Civil Service, concludes that "national needs" are being ignored by the educational establishment and that a more "open" approach is needed to ensure that those demands are satisfied.

The UGC should be replaced by a council with no more than 15 members, says the committee's report, while a newly created UK Education Commission would provide advice on the educational needs of industry and commerce.

The new bodies would have a membership that reflects national interests. The committee was emphatic that industry and commerce "Do not at present have a satisfactory focus for the expression of their views and on that account were not playing as full a part as they might in national debate on education".

The UGC, established in 1919, currently has 17 members but more than a dozen sub-committees. The proposed council would be steered principally through the new office of a director general, a full-time member who would preside in the chairman's absence. The profile of that chairman must also change. He or she must be "An eminent person from outside the academic world".

The constitution of the council would be changed dramatically with the membership no longer dominated by prominent academics. The UGC, presently chaired by Sir Peter Swinnerton-Dyer, has only one woman member — a fact that is singled out for criticism by Croham. A council dominated by men would be unlikely to explore how women could play a fuller role in universities, says the report.

While not overtly criticizing the present system, the nine members of the Croham committee conclude that "Through no fault of its present members, the UGC lacks the necessary balance of experience". The Croham report emphasized that the academic judgement frequently asked of the UGC should be conducted within the perspective of "public interest". There must be a wider economic and social perspective when addressing these questions.

The UK Education Committee which could either be assembled periodically or be in permanent session, would be responsible for advising the government "on national goals for the education service as a whole, and on any reforms which might be necessary". Both new bodies are to be assisted, if the recommendations gain approval, by triennial budgets established for the universities, assuming the annual inflation rate remains below five per cent.

The coordination of the management and funding of medical education also needs attention, says the Croham study. A sub-group led by Lord Butterworth, former vice-chancellor of Warwick University, specifically investigated the problems. The current machinery which depends on joint policies hatched by the Department of Education and Science, the Department of Health and Social Security, and the UGC is weak, says Croham, "with the result that too much is left to chance. That is a matter which needs urgently to be tackled by the departments concerned". **Bill Johnstone**

The Croham report is published at £7.20 as "Review of the University Grants Committee", Command 81, HMSO.

New technique to gauge sea ice

London

A NEW method, employing the characteristics of impulse radar, has been developed to measure the thickness of sea ice, allowing oil companies to determine easily the forces exerted by icefloes against structures such as oil or gas platforms.

The radar unit, developed jointly by Exxon Production Research Company and the British-based Cambridge Consultants, is mounted on a helicopter and emits short pulses of electromagnetic energy from antennas fitted on one side of the aircraft. The energy is in part reflected from the top of the ice and also from the interface between the bottom of the ice and the sea water. These reflections are collated by a receiving antenna housed at the other side of the helicopter.

The time difference between the received signals and the velocity of this radar energy as it travels in the ice allows the thickness to be easily determined. The device, which can accurately measure up to 30 ft to within one foot, supersedes manual drilling techniques. According to the inventors of the impulse radar device, "it is possible to collect more data in 30 minutes than can be obtained by a six-man crew drilling for two days". Bill Johnstone

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All change at the top for India's nuclear agency

New Delhi

THE crisis in India's Department of Atomic Energy (DAE; see *Nature* **325**, 565; 1987) has deepened with the resignation of Dr P.K. Iyengar, director of the Bhabha Atomic Research Centre, the physicist credited with the design of the plutonium device that India exploded in 1974. His departure follows the announcement on 9 February of the appointment of his rival, Dr M.R. Srinivasan, head of the Nuclear Power Board, as successor to Dr Raja Ramanna, the retiring chairman of the Atomic Energy Commission (AEC).

Iyengar, who is 55, said he was seeking voluntary retirement after 32 years of service. Dr N. Srinivasan, a close associate of Iyengar, has also resigned from his post as director of heavy-water projects but for reasons, he says, unconnected with the controversy over who should be the new chairman of AEC.

The Prime Minister, Mr Rajiv Gandhi, who is also in charge of atomic energy, is upset over the row among senior scientists at a time when the threat of a Pakistani nuclear device is looming large and when India is launching an ambitious 15-year programme to expand nuclear power capacity from 1,000 MW at present to 10,000 MW. In the wake of criticism in the media about the inept handling of the succession at AEC, Mr Gandhi personally intervened, but was unable to dissuade Iyengar from resigning.

The vertical structure of Indian scientific organizations and the immense power bestowed on their heads leads to intense competition for senior posts. The AEC chairman automatically becomes secretary to the government; he also draws the highest salary of any scientist.

M.R. Srinivasan, who will take over from Ramanna at AEC on 1 March, is a British-trained mechanical engineer. He joined DAE in 1955 and was in charge of its nuclear power projects division until, in 1984, this was reconstituted as the Nuclear Power Board, whose chairman he became. No successors to Iyengar and Srinivasan have yet been named.

In the meantime, Mr Gandhi has set up a committee under his science adviser, Professor M.G.K. Menon, to study the Soviet offer of two 440-MW light-water reactors. When the offer was made four years ago it was rejected by Ramanna and Iyengar because it did not fit the Indian nuclear programme. Following so soon after Mr Mikhail Gorbachev's visit to India, the prime minister's action is interpreted as a sign that India may now import the Soviet reactors. K. S. Jayaraman

More compensation in Finland for nuclear accident victims

London

FINLAND's Ministry of Trade and Industry is preparing an amendment to the law on nuclear accidents that will considerably increase the maximum compensation payable to victims. According to the Ministry of Justice, the maximum liability of nuclear power companies will probably be increased tenfold to Fmk 500 million (about £70 million) per station, and the maximum state liability by a factor of 2.5 to FM 1000 million. The bill has already been passed by the present parliament, but, having been introduced late in the parliament's four-year life, it will have to be resubmitted to the incoming parliament after the March general election. In the aftermath of Chernobyl, however, there seems little doubt that the bill will become law.

Although Finland escaped the worst of the first wave of Chernobyl fallout, the government's handling of the incident has been widely criticized. In Finland, the task of informing the public was left to the nuclear 'experts', in particular the head of the Institute of Radiation Protection, Ante Vuorinen, who said there was no information he could give except that radiation levels were up — but nowhere near the danger level. In fact, the radiation levels came close to the danger level only at the military base of Kajaani but the experts' attitude did not inspire public confidence. Doctors in particular complained that they had to try to cull information from the general press, which had frequently either misunderstood the little told them, or were reprinting foreign speculations.

Later studies of information flow and media coverage, made at the Universities of Helsinki and Tampere, compared the government's handling of the emergency unfavourably to the more open approach followed in neighbouring Sweden, though the Tampere study established that some nuclear experts felt it was unfair to leave the reporting of nuclear power issues in the hands of editors, most of whom openly admit to being opposed to nuclear power.

But despite this, the effect of Chernobyl on Finnish public opinion was less marked than might have been expected, the Tampere study concluded. Public opinion polls gave a level of 60 per cent opposed to nuclear power in May, compared with 33 per cent before Chernobyl. But this had fallen to 58 per cent by June and to 44 per cent by December. The time-scale, however, was significant — the accident came just as the government was about to give the go-ahead for a fifth nuclear power station in Finland, a scheme that has now been shelved.

Equally, however, post-Chernobyl demands that Finland should phase out the existing stations were summarily rejected. Apart from peat and 'energy woods', Finland has no domestic energy resources, and imported fossil fuels, including Soviet oil, are not only expensive but are also harmful to the country's main natural asset, the forests.

From the beginning of their nuclear power programme, the Finns have been mindful of the dangers. Two of their four existing nuclear stations were purchased from Sweden and two from the Soviet Union. The latter are of VVER-type — the RBMK (the type used at Chernobyl) has so far never been installed outside the Soviet Union.' The Finnish VVERs, however, have a feature not found in the Soviet prototype — a Westinghouse containment building (Finnish wits call the composite an 'Eastinghouse').

But, since Chernobyl, the main nuclear power threat has seemed, to many Finns, not their own 'Eastinghouses', but the other Soviet RBMK stations, at Ignalina and Leningrad, far nearer than Chernobyl. Finland has, of course, acceded to the new Convention on the Rapid Notification of nuclear accidents, drawn up by the International Atomic Energy Agency last October, and has also (two weeks ago) concluded a bilateral agreement on nuclear safety with the Soviet Union.

One idea now current in Finland is that this cooperation could include the construction by the Finns of containment vessels for the Ignalina and Leningrad RBMKs. The Soviet view is that containment buildings are unnecessary and could not have prevented the escape of radioactive material at Chernobyl. **Vera Rich**

Texas amongst early contenders in the race to subsidize the SSC

Washington

THE Department of Energy (DoE) has sounded the starting gun and the race to play host to the Superconducting Super Collider (SSC) is under way. On 10 February, Secretary of Energy John Herrington explained how and when a site for the SSC, which will be the largest particle accelerator ever built, will be selected. The prize for the state that wins will be construction jobs and a long-term increase in local spending.

Construction of the $4,400 million instrument is due to begin in 1989 and to be completed in seven years. Offers of sites will first be reviewed by a panel whose members will be selected by the National Academy of Sciences and the National Academy of Engineering. On the basis of the panel's recommendations, the DoE will select the best site by July next year, subject to later environmental and geological investigation.

Hot dog! The S.S.C. AND Mark Thatcher!
BIRCH

One of the considerations will be the guarantees given by bidders to help contain the cost. Herrington said last week that the DoE is assuming that the chosen state will have offered to provide the land on which the collider is to be built, but the bid will probably have to contain much more than that. One candidate site is that of Fermilab in Illinois, where the existing proton accelerator could serve as an injector for the new machine, perhaps saving a few hundred million dollars. At least two other states are said to have made offers to match that saving with cash.

Texas has been interested since the project was first discussed by the High-Energy Physics Advisory Panel early in the decade, while New York and California are among the others now interested. Overseas contributions may also help. Herrington said that he "wouldn't be surprised" if foreign contributions accounted for 25–50 per cent of the construction cost.

While the future of the SSC will rest ultimately with Congress, the DoE has not asked for new funds for the next fiscal year, beginning on 1 October, but assesses that the $35 million needed will be reallocated from existing funds. But winning congressional approval for the projected $348 million expenditure for the collider in fiscal year 1989 "will be a battle," according to one Senate staff member. The balance of the costs will have to be agreed by the next Congress.

The operating costs of the SSC are expected to be $270 million a year, and will be borne by the DoE. Herrington said that the funds would not be diverted from other programmes such as those at Fermilab and the Stanford Linear Accelerator Center, but a spokesman added later that those laboratories might not be operating when the SSC is on line.

Steven Dickman

Japan looks to sea for the extra acres and airports

Tokyo

JAPAN is expanding into the sea. Several multi-billion dollar projects to construct reclaimed islands off Japan's coast are on the drawing board and reclamation work for the new Kansai International Airport is already under way. This is a massive operation calling for the creation of a man-made island of more than 5 km^2 in Osaka Bay and connected to the mainland by a 4-km road and rail bridge. The airport is due to be completed in 1993 at a cost of Y1 million million ($6,600 million); a further Y2.5 million million will be spent on construction of railways, expressways, a residential area, parks, a water supply and a sewage system. Future plans call for the airport to be more than doubled in size to accommodate two additional runways.

The soft clay floor of the bay will pose problems for Japan's civil engineers. After ground improvement by sand compaction and drainage techniques, an 11-km wall will be built around the site before infilling with about 200 million m^3 of sand and soil derived from the mainland.

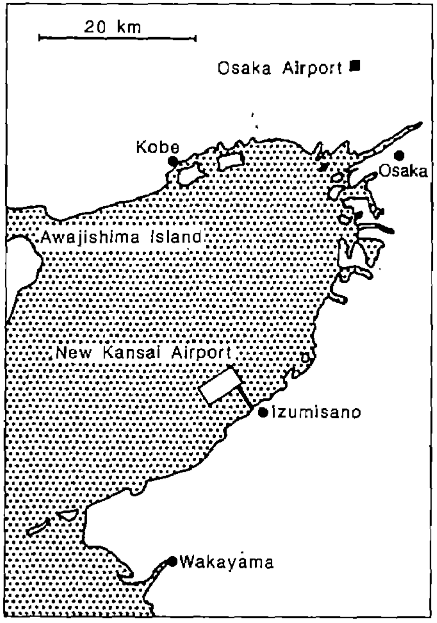
This is not the first or last man-made island of such a scale to be built by Japan. Port Island, of comparable size to the new airport, was completed off Kobe in 1981, and Rokko Island, its twin nearby, is nearing completion. Osaka plans to create two new islands in its harbour by dumping domestic and industrial garbage behind retaining walls. And Tokyo has the most ambitious plans of all — the National Land Agency has called for the reclamation of 17 km^2 along the waterfront of Tokyo Bay by 1995, and under a cooperative government-private venture a bridge and tunnel will span the 15 km of Tokyo Bay between Kawasaki and Chiba Prefecture via a couple of artificial islands. The price of mainland property has made this reclamation programme attractive.

When the national government recently asked Tokyo metropolitan government to construct a loop to link the city centre with the coastal redevelopment plan, officials were appalled to discover that a 1.35-km section between Toranomon and Shimbashi, although costing only Y2,000 million to build, would entail spending Y430,000 million to cover land purchase for the 40-km wide road, the equivalent of more than $2,000 million per kilometre of highway.

Redevelopment itself only tends to worsen the situation: when Tokyo announced plans to move the metropolitan government offices from the centre of town to a new site in Shinjuku on the outskirts, land prices in urban areas within 30 minutes' commuting distance of Shinjuku doubled in a few years to well over a million yen ($6,600) per m^2.

Land prices are not the only problem. Many old city areas lack the necessary infrastructure, roads and sewage systems, for redevelopment; Osaka may boast a bullet train, but it still has open sewers that overflow during the rainy season.

So offshore development has proved attractive. There new land can be created for as little as Y100,000 per m^2. The only objections come from local fishermen, who can usually be bought off with suitable compensation — for the new Kansai airport more than Y40,000 million was paid in compensation to the 3,400 fisherman of Hyogo and Osaka Prefectures. Few of these fisherman ever fish anywhere near the site, but in Japan a blanket approach to compensation is favoured to quell dissent among those most affected.



The new reclamation projects are joint government–industry ventures set up under a new law introduced last year to encourage private investment in public works. Main contractors are chosen under a designated bidder system, which means only select companies can bid for the project. Fifty per cent of the funding for the project is provided by the companies themselves, which buy low interest bonds from the local government.

The prospects for any foreign companies with plans to compete with local constructors seem slim. But following intense diplomatic efforts, the United States has managed to pick up at least one crumb from the Kansai airport contract. Bechtel Civil Corporation, a San Francisco-based engineering consulting firm, has won a Y30 million subcontract to survey facilities at international airports overseas. **David Swinbanks**

Export licence rules to ease?

Washington

IN response to the publication of a National Academy of Science report critical of US export control policy, the Department of Commerce is proposing significant changes in its export licensing procedures. The aim is to stop "the growing shift away from American products" without jeopardizing national security.

Secretary of Commerce Malcolm Baldrige announced the proposed changes last week. Although some need further approval, either from the White House or from Congress, others will go into effect immediately. This week, for example, the department is expected to publish in the *Federal Register* its revised plans for the general certified end user (G-CEU) licence. Dubbed the "gold card" scheme when it was first suggested last summer (see *Nature* **323**, 100; 1986), it provoked strong criticism from industry and has been substantially revised as a result. Now, G-CEUs will be awarded to companies, such as Fiat in Italy, controlled by CoCom (Coordinating Committee on Export Controls) governments. A second general licence would allow routine shipments to all agencies of CoCom governments. Future efforts will extend G-CEUs to other Western companies.

Another change expected to be welcomed by industry will be a loosening of the so-called parts and components regulations. At present, anything manufactured outside the United States containing controlled US parts still requires a US export licence if re-exported. The new regulations will require licences for products containing some fraction (rumoured to be 20 per cent) of US parts or components.

Boyd McKelvain, head of the Industry Coalition on Technology Transfer, says the Commerce Department's plans are a "tremendous step in the right direction". He says the plans to eliminate many controls on low technology items could have a large impact on trade. McKelvain says the large US trade deficit coupled with the National Academy of Science's report allowed industry's voice to be heard.

But the Department of Defense has not been overwhelmingly enthusiastic about the Commerce Department's proposals, even though the G-CEU scheme came originally from Deputy Undersecretary of Defense, Stephen Bryen. There is still concern that tough controls are needed to prevent the Soviet Union from gaining access to sensitive US technology. The Defense Department is preparing its own agenda for revising export controls, said a Pentagon spokesman. Ultimately, the National Security Council will have to balance the concerns. **Joseph Palca**

Accuracy, statistics and fraud

Sir—The catalogue of errors in scientific publications exemplified in the article by Stewart and Feder[1] will not surprise any statistician or biometrician, least of all one who has acted as a statistical referee of biological papers submitted for publication. Stewart and Feder refer to a paper[2], where "almost all the data ... are contained in three figures which cannot be reconciled with each other", and this experience could be mirrored only too often.

Biometricians, and the Biometric Society, have been concerned with this problem for many years, an early example being the critical review by Mead and Pike[3]. The British region of the society held a full-day meeting in December 1981 on "Statistical Presentation of Information in Biological Journals", where journal editors and statistical consultants put forward views that were discussed by a panel of experts and the audience. The *British Medical Journal* and the *Journal of Ecology*, representing very different areas of biology, were subjected to especial scrutiny. Although we believe the situation to have improved, there is still a long way to go. That the problem persists is shown by a letter[4] that you published in the same week as the article by Stewart and Feder, where a biometrician criticized a paper for a simple arithmetical error.

The problem is not that there may be different possible statistical interpretations of the same data. Certainly there are different statistical techniques available for analysing the same data, and indeed there is a proliferation of computer packages that vary considerably in their statistical reliability. A statistical technique that I would not advocate could appear in a paper submitted to me as a referee: I might be slightly unhappy but, provided the interpretation was correct, I should not recommend rejection of the paper. Too often, however, as in the example quoted by Stewart and Feder, incorrect statistics mean that a paper is actually wrong. There is no need to postulate outright fraud. Sometimes authors genuinely do not understand their results — and this usually means that they have either not sought statistical advice or not made proper use of it. At other times, research workers will do an experiment to test a hypothesis and then, consciously or unconsciously, ignore results that do not conform to their preconceptions. With the pressure to publish, there is a tendency to cut corners.

It is wrong to blame only authors for making misleading statements[1]. Too many referees are insufficiently critical, and indeed too many editors do not seem aware of their responsibilities. The position might well be improved if you, sir, and your fellow-editors used referees who are able to distinguish minor weaknesses from substantial errors caused by inability to use correct statistical methods. The consequence might be fewer papers published, but those accepted would be of higher quality.

G.H. Freeman

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Coventry CV4 7AL, UK

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2. *Ann. Surg.* **194**, 189–192 (1981).
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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

Sir—There is a serious methodological error in the paper by Stewart and Feder (*Nature* **325**, 207; 1987), for there is no control group. Their own publications, submitted to independent assessors, could have fulfilled this role.

A. M. W. Porter

37 Upper Gordon Road,
Camberley, Surrey GU15 2HJ, UK

Sir—Two recent papers have significant bearing on an important issue discussed in *Nature* recently (**325**, 207; 1987), namely the integrity of the scientific literature and specifically the problem of "scientific fraud".

In the first of these papers[1], de Lacey and his colleagues asked the question, "How accurate are quotations and references in medical journals?" Their depressing answer was — not very! "...the original author was misquoted in 15% of all references, and most of the errors would have misled readers. Errors in citation occurred in 24%, of which 8% were major errors — that is, they prevented immediate identification of the source of the reference."

In the second paper[2], I tried to assess the occurrence and the significance of 'self-confessed' errors in the biological literature, that is those instances where authors subsequently admit that something they had earlier published had not actually happened the way they originally said it had.

Again, the result of this survey was depressing. In a number of biological journals, including acknowledged leading journals, some 2 per cent of all papers are subsequently modified by their authors. While many of these modifications are primarily cosmetic, perhaps half of them are highly significant — 'we didn't actually use the buffer, drug, concentration, machines settings, number of animals,...' we originally said we did.

The number of such admissions may seem to be small, but how many such mistakes, all of which render impossible the repetition of the work as originally published, are not detected ... or not admitted ... or not published?

At question is a fundamental point, the 'accuracy' of the published paper; all of us — readers, reviewers, editors — have to believe that what was reported did in fact take place.

Fraud, that is, a deliberate intention to mislead, which is (it is to be hoped) only rare, will account for some inaccuracies. But it also seems that a lack of sufficient care may be widespread and is responsible for many more inaccuracies. We readily and rightly condemn the fraudulent, but the end result from the activity of the careless is equally damaging to the framework, the reputation and the progress of science. Should not the careless be equally condemned, and carelessness guarded against with a vigour equal to that with which we guard against fraud?

John R. Sabine

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1. de Lacey, G., Record, C. & Wade J. *Br. med. J.* **291**, 884–886 (1985).
2. Sabine, J.R. *Bioscience* **35**, 358–363 (1985).

Dissent in Singapore

Sir—I should like to point out some errors in the letter "Dissent disallowed in Singapore" by Louis Mahadevan (*Nature* **324** 298; 1986).

Firstly Mr J. B. Jeyaretnam was gaoled not for being "the voice of dissent in Singapore" but on a criminal offence. Jeyaretnam and Mr Wong Hong Toy were fined and gaoled for making a false declaration about their political party's accounts. Both Jeyaretnam and Wong appealed to the Privy Council, which dismissed the appeal with costs. Is Mahadevan questioning the integrity and independence of the Privy Council?

Second, Mahadevan alleged that the voice of dissent in Singapore is to be silenced at any cost. Far from it. Jeyaretnam has continued to voice his dissent during and since his gaol term through local as well as foreign publications circulated in Singapore.

Third, Mahadevan alleged that two publications were sanctioned for reporting Jeyaretnam's imprisonment and consequent loss of seat in Parliament. This is not true. The *Asian Wall Street Journal* was cited for contempt of the judiciary and apologized in court. *Time* magazine was sanctioned because it refused to publish corrections of errors in its article; it eventually admitted the errors and printed the corrections.

Mary Seet-Cheng
(Acting High Commissioner)

Singapore High Commission,
2 Wilton Crescent, London SW1, UK

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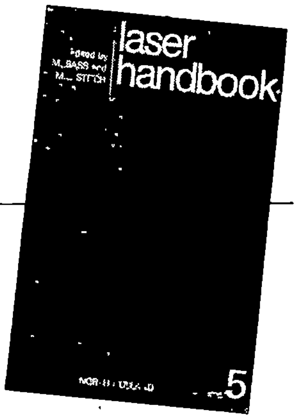

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Order for order–disorder?

There seems to be a simple rule for telling when some semiconducting ternary alloys have an order–disorder transition below the melting point. Will it apply to potentially commercial semiconductors?

CHEMISTRY at its most appealing is applied common sense. This is the spirit in which people in the nineteenth century were able to devise empirically based rules relating properties of one kind to those of another for some class of chemicals. Sometimes the outcome would be simply a useful way of codifying data and of making plausible predictions, as in the empirical statement that the entropy of vaporization at the boiling point is roughly constant, called Trouton's rule. But in the early decades of the century, much bigger prizes had been won from these empirical relationships. Would there have been an atomic theory in Dalton's time without the law of multiple proportions, for example?

So it is good that the tradition still flourishes, even in unlikely connections. Thus Alex Zunger, from the Solar Energy Research Institute at Golden, Colorado, has just applied exactly such a technique to the codification of order–disorder transitions in the three-component alloys on which those who design new semiconductors have set their hearts (*Appl. Phys. Lett.* **50**, 164; 1987).

The importance of the problem is evident. Sooner or later, no doubt, silicon will be replaced by materials such as gallium arsenide (GaAs) or indium antimonide (InSb) in the microcircuits on which we all rely. The principle, which explains why chemists are closely engaged with these developments, is simply that if, say, germanium is a semiconductor, an alloy of the two elements on either side of it in the Periodic Table, Ga and As, will almost certainly be a semiconductor as well. Experiment indeed confirms that the outer-shell electrons of Group III gallium and Group V arsenic fill the valence band of an equi-atomic alloy, but leave the conduction band essentially empty.

For the past decade, chemists have been busily ringing the changes on this and other recipes for making semiconductors, knowing how great the practical benefits could be. GaAs should be faster than silicon, while sandwiching an alloy in which 30 per cent of the Ga atoms are replaced by Al (also in Group III) between slices of GaAs will make an infrared laser as well. The practical snag is the difficulty of controlling the stoichiometric balance of the composition to a few parts in 100 million, the level at which electronic doping by foreign ingredients can be significant.

Zunger is concerned with a lesser but still important snag — the circumstance that semiconductors that are ternary compounds should in principle exist in one or other of two alternative states, ordered and disordered. In the case of $Al_xGa_{1-x}As$, for example, where Al and Ga are electronic substitutes for each other and where the electronegativity of the As is likely to give it anionic properties and thus large size (relative to the Al and Ga atoms in the lattice), it is an interesting but open question whether the Al and Ga atoms are distributed randomly and, if not, whether they are ordered in clusters or in some other way. Moreover, the electronic properties of the material could depend on what the answer is. The transition from order to disorder should decrease the band-gap, for example.

Luckily, most semiconductors appear to be built on the architecture of the diamond lattice — that of a tetrahedron in which an anionic atom is surround by four cationic atoms (and, for that matter, vice versa), which allows Zunger plausibly to compare semiconductors whose order–disorder transitions are likely to occur at low temperatures where they cannot easily be observed with those of other materials where the transition has actually been observed. Specifically, he considers the range of ternary alloys or molecular compounds in which two electronegative Group V or VI atoms are combined respectively with one Group I and one Group IV atom or with one Group II atom and one from Group V. $AgInTe_2$ might stand for the first class of compounds $ZnSIAs_2$ for the second. The materials are respectively chalcopyrites and pnictides. Zunger lists more than 30 known semiconductors of both kinds.

These materials differ from those at the centre of the device-makers' attention in that the cationic components, not being isovalent, are far from being electronic substitutes for each other, as Ga and Al are. Zunger points to two energetic consequences of this state of affairs for the order–disorder problem.

First, the ordered state in which each anionic atom (from group V or group VI) is surrounded tetrahedrally by two pairs of cationic atoms is likely to persist to much higher temperatures than in alloys in which, say, Ga and Al atoms may substitute for each other with negligible energetic penalty. This is what the experiments show: order–disorder transitions have been observed in 13 out of the 30-odd chalcopyrite semiconductors at temperatures not far short of the melting point (about 1,000 °C.) The snag, for materials such as $Al_xGa_{1-x}As$, is that the energetic differences between the cationic components are so small that the transition, if it occurs, will be at too low a temperature for it to be practicable to tell whether the usually disordered state of the alloy is the lowest or merely a metastable state.

Zunger's second observation is where the common sense comes in. If there are low-temperature ordered states in ternary alloys of this kind, they will not be tetrahedrally symmetric about the cations, but distorted so as best to accommodate the two different ionic radii of the two pairs of cations. The disordered state, on the other hand, in which cations are distributed at random about their central anions, must be tetrahedrally symmetric, which means that the lattice embodies a substantial penalty in internal energy (or in enthalpy) in exchange for the benefits of the extra entropy that comes from the disorder.

What Zunger does is what chemists have been doing for two centuries. He defines a parameter (called u) to represent the measured departure from tetrahedral symmetry in the ordered phases of the chalcopyrite alloys. (Numerically, u is determined by the difference, in the ordered phase, between the distances from the central anion to each of the two different kinds of cations.) Intuitively, one would expect that if there are ternary alloys that make a transition from order to disorder before they melt, they will be the alloys for which the value of u is the smaller.

This, magically, is how it turns out. For the 23 compounds for which data exist, the 13 that make a transition to disorder before melting have u less than 0·265; the remaining ten, which remain ordered up to the melting point, have larger values of u. Emboldened by the fact that there are no known exceptions to his rule, Zunger goes on to predict which side of the fence ten compounds not yet made will fall.

There are several lessons to be learned from this elegant exercise. The argument that lattice strain may stabilize order may be extended to alloys of more immediate interest. It may even be possible to make a numerical connection between disorder and the reduction of the semiconductor band-gap it brings about. But the chief may be that there is mileage yet in a style of argument that seemed to go out of fashion when people took to chemistry by supercomputer.

John Maddox

Alzheimer's disease

Progress in molecular pathology

Brian H. Anderton

SENILE dementia of the Alzheimer type is believed to affect 5 per cent of people over 65 years of age. That the disease is a major health problem is reflected by the intense research activity aimed at elucidating the pathogenesis and cause of the disease. The techniques of molecular biology are now being brought to bear on this problem; and one of the first results is reported in a paper by Müller-Hill, Beyreuther, Masters and collaborators on page 733 of this issue[1].

Senile plaques and neurofibrillary tangles are characteristic of the histopathology of Alzheimer's brains. Two abnormal filamentous deposits are associated with these lesions and determination of the chemical composition of the filaments may provide clues about the underlying molecular pathology. The filaments that comprise the neurofibrillary tangles, paired helical filaments (PHFs), appear in the electron microscope to consist of two 10-nm wide filaments twisted about each other in pairs[2]. PHFs are intracellular and, as well as being present as tangles in neuronal perikarya, are found in many of the abnormal dendritic and axonal processes, or neurites, that make up the periphery of the senile plaque. The senile plaque, an area of disorganized neuropil up to 150 µm across, often has an extracellular core which is a deposit of amyloid. This cerebral amyloid is ultrastructurally distinct from PHFs, consisting of 4–8-nm wide filaments that are not wound together in pairs[3]. Plaques and tangles can be widespread in several cortical areas, particularly in the hippocampus, of Alzheimer's brains and their numbers are related to the severity of dementia.

Cores

The cores of extracellular plaques have been isolated in several laboratories[4-8]. There seem to be at least two components to the plaque core, an inorganic aluminosilicate[9] and an abundant polypeptide of relative molecular mass (M_r) about 4,000, termed A4 and isolated previously by Masters *et al.*[5]. These authors obtained a partial amino-acid sequence for A4 and showed that it is probably identical or closely similar to the amyloid protein that Glenner and Wong had already isolated from cerebral blood vessels from patients with Alzheimer's disease and Down's syndrome and which the latter authors had also sequenced[10]. Immunohistochemical studies[7,11,12] confirm that plaque-core A4 is related to vascular amyloid protein. The paper by Müller-Hill and his co-workers in this issue[1] reports the isolation and sequence of a complementary DNA clone that seems to encode a much larger precursor protein of the amyloid A4 peptide and the authors speculate that A4 is derived from a membrane glycoprotein. They also report the complete amino-acid sequence of A4, which is either 42 or 43 residues long.

Müller-Hill's group screened a human fetal brain complementary DNA library with a synthetic oligonucleotide based on part of the A4 amino-acid sequence. The sequence of a 3,353-base pair apparent full-length DNA shows that the A4 sequence is within a much larger one encoding a protein of 695 amino-acid residues and of M_r about 79,000. The amino terminus of the long precursor begins with a signal sequence for translocation of the polypeptide through the endoplasmic reticulum and is followed by a cysteine-rich region up to residue 187, suggesting a disulphide-stabilized domain. Next there is a 100-residue segment rich in acidic side chains which the authors speculate may be involved in the electrical activity of cells. Two potential *N*-glycosylation sites run from amino-acid residues 467 to 469 and 496 to 498 and a hydrophobic sequence characteristic of a transmembrane domain runs from residues 625 to 648. The A4 sequence is between residues 597 and 638 and is therefore partially included in the putative transmembrane region. Overall, the A4 precursor has the characteristics of a membrane-spanning glycoprotein.

Alzheimer's brains and normal adult human cortex contain two transcripts of 3.2 and 3.4 kilobases, respectively, detected by Müller-Hill and co-workers[1] using their clone. Thus, the gene encoding the A4 sequence is not uniquely expressed in Alzheimer's disease. Gajdusek reported recently (*4th meeting of the International Study Group on the Pharmacology of Memory Disorders Associated with Aging* Zurich, 16–18 January 1987) that his group has independently isolated[13] another human complementary DNA for the A4 precursor and shows that the gene is expressed in thymus, muscle and heart as well as brain but not in liver or placenta.

Gajdusek also reported that the gene is highly conserved because positive Southern blots have been obtained with genomic DNA from hamster, mouse, rat, chicken, lobster, *Drosophila* and yeast. The next stage will probably be to establish the tissue and subcellular distribution of the A4 precursor in healthy humans and in Alzheimer's disease and to try to determine how A4 is generated and deposited as amyloid in diseased brain.

Müller-Hill's group shows[1] and Gajdusek confirms[13] that the A4 gene is located on human chromosome 21. This result is particularly interesting because trisomy of chromosome 21 results in Down's syndrome. Individuals with Down's syndrome develop a brain pathology indistinguishable from Alzheimer's disease by the time they become 60 years old. Because of this association with Down's syndrome, it has been thought for some time that the common form of Alzheimer's disease, which is not exclusively sporadic but often shows a familial tendency, might be linked to a gene on chromosome 21 that would cause Alzheimer's disease either by a gene-dosage effect (Down's syndrome) or as a result of a mutation (Alzheimer's disease). A much rarer form of Alzheimer's disease (familial Alzheimer's disease, FAD) is determined by a single autosomal dominant gene and, in carriers, leads to an earlier onset of dementia. St George-Hyslop reported in Zurich on restriction fragment length polymorphism analysis of four families with familial Alzheimer's disease. Using several markers for different regions of chromosome 21, he found that the FAD gene is possibly in the proximal region of the long arm of chromosome 21. This should not be taken as definitive as more pedigrees need to be investigated, and it is premature to assume that the A4 precursor gene is the Alzheimer's gene, although this is an intriguing possibility.

Relationship

What of the relationship of A4 to the PHF proteins in the tangles? Masters and Beyreuther have previously proposed[14] that PHFs are also composed of A4 but that A4 is assembled in a different way from that in amyloid fibrils, possibly with some of the amino-terminal residues proteolytically removed; this model is largely based on their isolation and sequencing of A4 from fractions enriched in PHFs. However, other studies with antibodies against either PHFs or plaque and vascular amyloids fail to demonstrate cross-reactivity between the two filamentous proteins and do not confirm their hypothesis. One possible explanation for this discrepancy is that PHF preparations are contaminated by amyloid and, hence, the A4 in isolated PHFs does not originate from the filaments but is from amyloid[7]. Beyreuther (*Dementia and Stroke. Biology of the Aging Brain* Heidelberg, 18–20 September 1986) and Gajdusek in Zurich both reported that A4 is extracted from PHF fractions isolated from brain tissue of cases of the Guam Parkinson–dementia complex. Because neurofibrillary tangles without plaques are the hallmark of this disease, these authors argue that A4 must be a genuine component of PHFs and does not come from contaminating amyloid.

Other investigations[16-23] have shown

that certain antibodies against neurofilaments and against microtubule-associated proteins, particularly tau, label PHFs. Microtubule-associated tau, and possibly the neurofilaments that are incorporated into PHFs, may be abnormally phosphorylated. It has been reported[24] that tau protein isolated from Alzheimer's brains is defective in promoting microtubule assembly because it is abnormally phosphorylated. Davies and Wolozin reported (*16th American Society for Neuroscience* meeting, Washington DC, November) that a polypeptide of M_r 68,000 that is expressed at elevated levels in many neurons in Alzheimer's disease[25] may be a protein kinase.

But there are no substantiated reports that the structural protein of microtubules, tubulin itself, is found in PHFs and the neurofilament proteins in PHFs seem to be derived proteolytically from the side-arms that crosslink neurofilaments to one another and to other organelles. Thus, whether or not A4 is complexed with the cytoskeletal components of PHFs, it will be important to see if the post-translational changes that produce A4 from its precursor, possibly located in the plasma membrane, are similar to those that affect the cytoskeleton. The obvious candidates at the moment for the agents bringing about these changes are protein kinases and proteases. ☐

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Physical chemistry

Long-lived superoxide radicals

Martyn C.R. Symons

ALTHOUGH radicals trapped in matrices have been studied for many years, trapping during the process of gel formation is described for the first time elsewhere in this issue by J. Ragai (*Nature* **325**, 703–705; 1987). The superoxide radical-anion, of major importance in the biochemistry of oxygen, is trapped by this procedure, and Ragai shows that it has characteristic electron spin resonance and infrared spectra that indicate a strong interaction between the anion and the gel. No doubt this interaction contributes considerably to the fact that these species remain trapped for long periods, even after heating to 110 °C.

To the chemist, the term 'radical' implies a molecular fragment containing an unpaired electron, which can often be viewed as a broken bond as implied in the following reaction

$$A\upharpoonleft\!\downharpoonright B \xrightarrow[\text{and so on}]{\text{heat, light}} A(\uparrow) + B(\downarrow)$$

where $\upharpoonleft\!\downharpoonright$ symbolizes two paired electrons. These radicals, normally written as $A^{\cdot}$ and $B^{\cdot}$, are often very reactive and can rarely be isolated. But they can be stabilized by trapping in solids, their reactivity being lost because of their immobility (Fig. 1). Only when the solid is heated can these radicals regain their mobility and hence react.

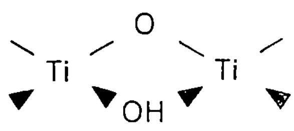
Ragai uses two techniques to establish the presence of radicals, infrared spectroscopy, which is non-specific, and the less well-known but extremely powerful technique of electron spin resonance spectroscopy, which is completely specific to species containing unpaired electrons. Hence, only radicals trapped in a matrix can produce an electron spin resonance spectrum — the matrix itself will be devoid of spectral features. Thus, electron spin resonance spectroscopy, which is extremely sensitive, can be used to detect the 'fingerprint' radicals even in very low concentrations.

Radical-cations and radical-anions differ from neutral radicals (such as the generalized species $A^{\cdot}$ and $B^{\cdot}$ in the reaction above) not only in their charge, but in their often reduced tendency to react together. The superoxide ion ($O_2^{\cdot -}$) is no exception, being much less reactive in the sense of bond making and breaking, than its conjugate acid $HO_2^{\cdot}$. Nevertheless, superoxide is sufficiently reactive and dangerous to biological systems that there is a natural enzyme, superoxide dismutase, specifically to keep the concentration as low as possible.

Superoxide ions are readily formed from oxygen by electron addition, but can also be generated by the Ti(III)–H_2O_2 system used by Ragai in the following reactions, where Ti(III) acts as a powerful electron donor and H_2O_2 reacts by undergoing bond fission, the electron staying with one half to form the stable hydroxide ion, leaving the other as the highly reactive $^{\cdot}OH$ radical. This is not detected as such by electron spin resonance, but readily extracts a hydrogen atom from H_2O_2 to give $HO_2^{\cdot}$. In neutral aqueous systems this largely ionizes to give $O_2^{\cdot -}$

$$\mathrm{Ti(III)} + H_2O_2 \rightarrow \mathrm{Ti(IV)} + OH + {}^{\cdot}OH$$
$${}^{\cdot}OH + H_2O_2 \rightarrow H_2O + HO_2^{\cdot}$$
$$HO_2^{\cdot} + H_2O \rightleftharpoons O_2^{\cdot -} + H_3O^{+}$$

Ragai's technique makes use of the fact that Ti(IV) readily forms highly crosslinked gels comprising chains with



linkages as a backbone, and it seems that $O_2^{\cdot -}$ (but probably not $HO_2^{\cdot}$ or $^{\cdot}OH$) can be trapped therein, in remarkably stable

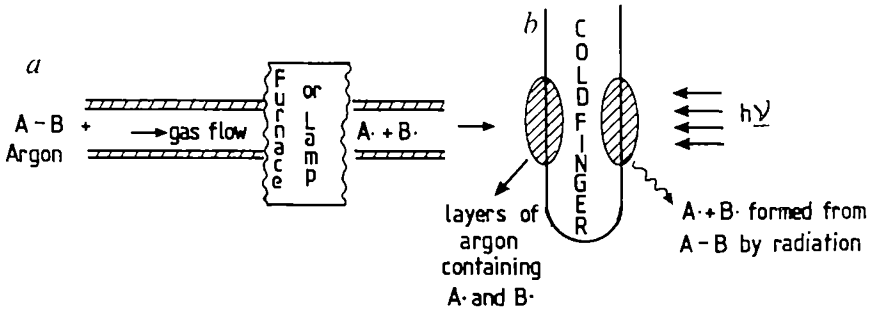

a
A – B +
Argon
gas flow
Furnace
or
Lamp
A· + B·
b
COLDFINGER
hν
layers of argon containing A· and B·
A·+B· formed from A – B by radiation

Fig. 1 Typical examples of isolating and thereby stabilizing reactive radicals by trapping in solids (matrix isolation). *a*, $A^{\cdot}$ and $B^{\cdot}$ radicals formed in flowing inert gas by thermolysis or photolysis followed by rapid solidification on a liquid helium cold finger. *b*, The same radicals generated *in situ* by photolysis or ionizing radiation (of energy $h\nu$).

sites. Thus they withstand heating to 110 °C and remain unchanged after 2 years at Egyptian temperatures.

Two aspects of these results indicate the nature of these sites. One is the electron spin resonance spectrum and, in particular, the low value of $g_{\max}$, g being a measure of any orbital magnetic contributions. To explain the significance of this, recall that 'free' $\mathrm{O_2^{\cdot -}}$ has its unpaired electron in an unusual orbital in that it rotates around the axis of the dioxygen unit, giving a maximal orbital contribution. This rotation makes it impossible to detect $\mathrm{O_2^{\cdot -}}$ by normal electron spin resonance. It is possible, however, to quench this orbital rotation by various interactions. A revealing experiment involves electron addition to normal oxygen dissolved in an alcohol which has been frozen to about 4 K in liquid helium. (The electrons were generated by ionizing radiation: Symons, M.C.R. & Stephenson, J.M. *Trans. Faraday Soc.* **1,** 1579–1583; 1981.) No spectrum for $\mathrm{O_2^{\cdot -}}$ ions could be detected at 4 K, but on warming to about 77 K a well-defined spectrum was obtained, characteristic of fully solvated $\mathrm{O_2^{\cdot -}}$ ions. The key to this result is selective hydrogen bonding or solvation by the alcohol which is confined to one plane, thereby preventing orbital motion (Fig. 2). Even so, the resulting value of $g_{\max}$ is about 2.08, not 2.00, the value expected if orbital motion were impossible. Now, $\mathrm{O_2^{\cdot -}}$ in the $\mathrm{TiO_2}$ gel system has $g_{\max} = 2.02$, which is far closer to 2.00, and hence some sort of bonding which is stronger than normal solvation has to be invoked. This is almost certainly bonding to a Ti(IV) centre, as, for example

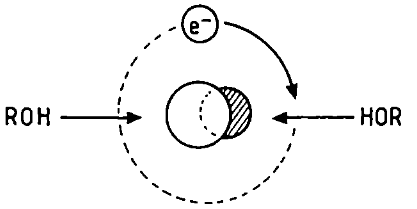
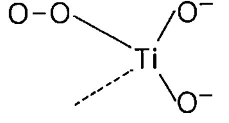


Fig. 2 Effect of hydrogen bonding from ROH molecules on the orbital motion of the unpaired electron in $\mathrm{O_2^{\cdot -}}$.



(1)

(If this idea is correct, then an extra fingerprint should be detectable at high instrument gain. That is, electron–nuclear hyperfine coupling to the low-abundant isotopes of titanium having magnetic nuclei. This cannot be seen in the published spectrum, but might be detectable at high gain.)

Further evidence for a structure of this sort comes from Ragai's infrared results. Free $\mathrm{O_2^{\cdot -}}$ ions do not absorb infrared radiation because of their high symmetry, but the O–O bond in unit (1), being asymmetrical, is expected to absorb, as was indeed the case. If this is correct, the species is perhaps better described as a peroxy-titanium radical rather than a superoxide ion.

It has only recently been realized that radical trapping has been occurring in nature for many millions of years. Most hard, non-metallic, geological materials, such as flint or calcite, form radical species which are permanently trapped within the solid on exposure to ionizing radiation. As there is always a low level of natural radiation, these trapped species are formed naturally and their concentrations gradually increase. Electron spin resonance spectroscopy can often be used to detect, characterize and estimate the yields of these centres (see, for example, Robins, G.V., Seeley, N.J., Symons, M.C.R. & McNeil, D.A.C. *Nature* **276,** 103–106; 1978). Also, many flints have been shown to contain trapped methyl radicals ($\mathrm{CH_3^{\cdot}}$) which are normally extremely reactive (Griffiths, D.R. *et al. Nature* **300,** 435–436; 1982). ☐

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T-cell receptors

Who needs more?

Ellis L. Reinherz

THYMUS-derived (T) lymphocytes comprise approximately 60–80 % of peripheral blood lymphoid cells and serve as both effectors and regulators of the vertebrate immune system. Unlike B lymphocytes, which produce immunoglobulin molecules acting to neutralize antigen in solution, T lymphocytes express membrane-bound receptors that detect perturbations in normal cell surfaces consequent upon invasion by viruses, certain bacteria and many intracellular parasites. Therefore, the elucidation of the structural basis for T-cell recognition is of basic theoretical and practical significance. On pages 683 and 689 of this issue[1,2], two groups report on the definitive identification of several additional types of T-cell receptors that differ in certain fundamental ways from the now classical T-cell receptor described three years ago[3–6].

Although this new set of receptors represents only a minor portion of peripheral T-lymphocyte receptors (less than 5 %), their early appearance in ontogeny and apparently broader specificity than conventional T-cell receptors, together with the absence of any obvious requirement for major histocompatibility complex (MHC)-restricted recognition (see below), thus defines a set of naturally occurring receptor variants that may provide insights into basic principles of T-cell receptor recognition in general. To put these findings in perspective, I shall first briefly review some of the salient features of T-cell recognition and its known structural basis.

In both human and murine systems, the subset of T cells that turns on the immune response, helper T cells, and the T cells responsible for specific destruction of target cells, cytotoxic T lymphocytes, express a T-cell receptor that is a molecular complex comprising at least five polypeptide chains[7,8]. Two of these are the highly variable disulphide-linked Ti α and β subunits, each encoded by a gene assembled by means of somatic rearrangement from pools of separate gene segments[9], as with immunoglobulin genes (see Fig. 1 and legend); together these two chains mediate antigen recognition. They are non-covalently associated with the three monomorphic CD3 subunits that are believed to mediate signal transduction when the T cells are activated by receptor binding. All these chains have been cloned and sequenced (see ref. 10 for review).

A fundamental feature of most CD3–Ti $\alpha\beta$-type T-cell receptors is that they recognize antigen only when it is associated on the surface of a cell with a molecule encoded by the MHC[11]. Thus, recognition of either a specific MHC molecule or nominal antigen alone will not be sufficient to trigger activation of a given T lym-

Note on nomenclature

In the interests of consistency, *Nature* will in future use the CD nomenclature for all T-cell differentiation antigens between CD1 and CD8. Some of the alternative designations for man and mouse are given in the table.

Preferred name	Man	Mouse
CD1	T6/Leu 6	TL
CD2	T11/Leu 5	—
CD3	T3/Leu 4	—
CD4	T4/Leu 3	L3T4
CD5	T1/Leu 1	Ly-1 (Lyt-1)
CD8	T8/Leu 2	Ly-2,3 (Lyt-2,3)

The CD usage has thus been imposed on this News and Views article as well as the papers to which it refers. This convention will however not be extended to antigens whose alternative designations convey information about the function of the molecule: the interleukin-2 receptor, for example, should not be called CD25. ☐

phocyte. This so-called MHC restriction is 'learned' or selected for during T-lymphocyte ontogeny in the thymus[12,13] and probably does not represent a structural limitation of the Ti αβ heterodimer, as it is possible for some Ti αβ heterodimers to recognize target structures without binding to MHC[14]. What then are the nature and function of the 'alternative' types of T-cell receptor?

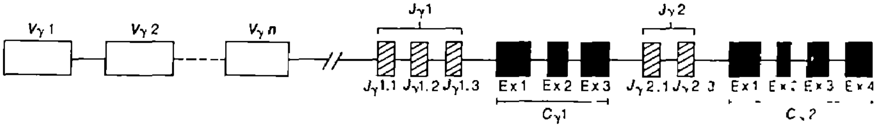
The new receptor complex

Several months ago, four groups independently reported on the identification of a CD3 complex that did not contain the Ti αβ heterodimer. The complex was detected on cell lines established from immunodeficiency patients[15]; clones established from fetal blood[16] and thymocytes[17]; and a leukaemia T-cell line[18]. All these cells express CD3 in association with a dimer containing a product derived from the γ gene, a gene essentially similar in structure to those encoding the antigen receptors of T and B cells, although with significantly more limited variability than α or β[19]. This was the first direct evidence that the γ gene encoded a surface receptor. Unlike the genes encoding the α and β chains, whose products had been identified as variable cell-surface molecules responsible for specific target recognition by human T cells[20], the γ gene was isolated from murine DNA in the absence of any evidence on its product, and its identification as a receptor gene rested largely on its structure and the fact that it is rearranged during T-cell ontogeny (see Fig. 1 and legend).

One obvious question was therefore why, if the γ gene encodes a variable surface receptor for antigen, no γ chain had ever been identified by the techniques successfully used to isolate the α and β chains. A possible answer is provided by estimates of the proportion of cells in normal lymphoid tissue that express the alternative receptor, which seems to be present on only about 3 % of human peripheral T cells and as few as 0.2–0.9 % of neonatal human thymocytes[21].

Moreover, almost all of these cells lack the CD4 and CD8 markers that characterize mature functional T cells (see Fig. 2 and legend; rare exceptions express CD8). This apparently very immature phenotype raises the question of whether these cells have a normal function in the mature immune system and has refuelled speculation, based on earlier observations in mice, that γ might instead have a special role early in ontogeny. Because γ gene transcription is particularly pronounced in immature T-lineage cells, such as fetal thymocytes, Tonegawa and colleagues proposed that the γ protein might associate with the β chain to give specificity for self-MHC, and thus allow for selection of self-MHC restricted cells during thymic ontogeny[22]. More recent analyses suggest a rather different role for γ in T-cell ontogeny (see later); and although a role in thymic education cannot be excluded, the proposal originally formulated by Tonegawa and co-workers is almost certainly incorrect in the light of what is now known about the biochemistry and pattern of expression of the γ receptor complex.

Fig. 1 Genomic structure and organization of the human Ti γ gene[23,29,30]. The organization of the γ gene is like that of all the known genes encoding lymphocyte receptors for antigen, in that it comprises separate segments that are brought together in the course of lymphocyte ontogeny by DNA rearrangements. In all cases, there are relatively few invariant or constant segments (*C*) that anchor the receptor in the membrane, and sometimes mediate an effector function, and a linked pool of two or three additional segments that together encode the variable (V) region of the receptor that recognizes antigen. The largest of these segments is the *V* segment, of which there may be a very large and diverse pool; in the course of ontogeny one of these *V* segments becomes joined to one of a smaller number of *J* segments. (Some receptor chains also have a *D* region, encoded by a further pool of segments in between the *V* and the *J* pools.)



The human γ locus[29-31] contains two constant regions, $C_\gamma1$ and $C_\gamma2$, each with several J_γ segments, as well as at least nine *V* segments that can recombine independently with either C gene. Although the human $C_\gamma1$ segment, like both of the mouse C_γ segments, has three exons (Ex), in the human $C_\gamma2$ segment the second exon is duplicated and both copies have lost the codon for the cysteine residue thought to be involved in interchain disulphide bond formation. In PEER and IDP2 cells, which have the non-disulphide-linked receptor, complementary DNA analysis suggests that the $C_\gamma2$ segment is used (ref. 32 and Krangel and Brenner, personal communication). There seems to be some variation in the duplicated $C_\gamma2$ exons from different cell lines[33] but whether these differences are due to distinct $C_\gamma2$ alleles, alternative RNA splicing or utilization of other C_γ segments yet to be defined is not known. (In mouse, by contrast, both $C_\gamma1$ and $C_\gamma2$ encode cysteines.)

The nature of the receptor

It is clear from the two reports in this issue[1,2] that there are at least two forms of the 'new' receptor. Borst and colleagues[1] report a disulphide-linked dimer of relative molecular mass (M_r) 70,000–80,000 (70–80K) associated with CD3 and reacting with anti-γ antibodies on the surface of cloned human peripheral T cells. After separation of the chains in reducing conditions, the γ component resolves into two bands at 40 and 36K. These have the same isoelectric focusing point and reduce to a single major component after endo-F treatment, and so seem to represent differently glycosylated forms of the γ gene product[1]. Borst *et al.* also detect a third component of M_r 43K that does not react with anti-γ antisera and that presumably forms a heterodimer either with the 40K or with the 36K γ chain.

This means that the receptor they now report is significantly different from the one originally described by Brenner and Krangel[15]. That receptor was a heterodimer from a cell line (IDP2) derived from an immunodeficiency patient and containing a 55–60K γ chain non-disulphide-bonded to a 40K non-γ product; they now find[2] a similar non-disulphide-linked form of the receptor on the leukaemic cell line PEER. This is clearly distinct from the receptor described by Borst *et al.*, both in the absence of the disulphide bonding and in the molecular mass of the non-γ component; however, Brenner and Krangel also report, on a human T-cell clone, a disulphide-linked version containing 36K and 40K γ components as described by Borst *et al.* For reasons that are not clear but are probably trivial, they do not detect the 43K component on their gels; but they postulate a second, non-γ component because of the very different migration of the CD3-associated bands in isoelectric focusing gels under reducing and non-reducing conditions. Brenner and Krangel have termed the non-γ component in these receptors δ; but because its relationship to non-γ components described by Borst *et al.* and other workers is unknown, I shall here refer to all non-γ components as X (see Fig. 2).

Whatever the nature of X, it is clear on grounds of disulphide linkage and of the molecular mass of the γ component that there are two distinct forms of the receptor. Moreover, the discrepancy in molecular mass of γ products in the non-disulphide-linked and disulphide-linked forms cannot be explained away by differences in glycosylation: endoglycosydase digestion reveals a substantial core protein size difference[2]. In fact, there is now strong evidence that both the size difference and the absence of disulphide bonding can be explained by differential usage of the two human C_γ gene segments (see Fig. 1): $C_\gamma2$ usage correlates with the expression of the non-disulphide-linked CD3-associated form, whereas $C_\gamma1$ usage results in formation of the disulphide-linked Ti γX heterodimer. Both forms appear in peripheral blood lymphocytes from normal humans. At this stage it is impossible to guess at the functional significance of the distinct forms — especially as mice do not seem to produce the non-disulphide-linked form, having cysteines in both $C_\gamma1$ and $C_\gamma2$.

What is the alternative receptor for?

Although the definitive word is not yet in, there is evidence that both forms of the CD3–Ti γX receptor mediate non-MHC-restricted killing of target cells. First, Borst *et al.* report[1] that cells bearing the disulphide-linked receptor display cytotoxic activity against a wide array of target cells. Second, Krangel and Brenner have data of a similar kind for clones bearing either the disulphide-bonded or the non-disulphide-bonded receptor and find that cytotoxicity is not blocked by antibodies against MHC gene products. This sets them apart from the classical cytotoxic T lymphocytes which must recognize MHC products in order to kill, and places them in a rather broadly defined category of lymphocytes with generalized cytotoxic activity that does not (again unlike that of classical cytotoxic cells) require prior sensitization and that is directed against a broad range of targets. Such killing may be targeted by antibodies bound to the surface of a cell (antibody-dependent cell-mediated cytotoxicity, or ADCC); or not, in which case it is called natural killing. Most 'natural killer' cells (possibly all) are also capable of ADCC: all the clones tested by Borst *et al.* show both activities.

Because it is possible to elicit a more-or-less indiscriminate wide range of cytotoxic responses from such T-lineage cells in the absence of identifiable receptor binding, it is a little difficult to know what to make of the broad-spectrum cytotoxicity of the γX-bearing cells. There is, however, some direct evidence, from studies by Moingeon *et al.* reported in this issue[23], that the CD3–Ti γX complex is a receptor for non-MHC-restricted recognition. These authors have produced specific antibodies against a T-cell clone (F6C7) which expresses a disulphide-linked receptor containing γ bonded either to another γ or to some other chain; and they are able to show that such antibodies can induce proliferation and the production of interleukin-2 by these cells, or block killing, depending on the conditions. That γ-bearing cells can kill in the absence of MHC recognition is not however a powerful argument against a more specific MHC-restricted cytotoxic function for at least some of them. Recall that CD3–Ti $\alpha\beta$ receptors can recognize antigen in the absence of MHC in the case of certain natural killer clones[14]. The prominent non-MHC restricted cytotoxicity documented in recent reports may be more a consequence of the ease with which immunologists can detect non-MHC-restricted killing than any limitation of the recognition repertoire of the CD3–Ti γX receptor.

Although functional and biochemical analyses have proceeded more rapidly in human systems, studies on the ontogeny of γ expression have made more progress in mice. The character of the receptor and of the cells that bear it are essentially the same in mouse as in man. A 35K γ chain, disulphide-linked to a 45K partner associated with CD3, is found on cells not expressing either of the murine equivalents of CD8 (L3T4) and CD4 (Ly 2), and the cells can be stimulated to proliferation and cytotoxicity by interleukin-2 and antibodies against CD3 (refs 24–27). However, it has been possible to show clearly in mice that the Ti–γX complex is predominantly a fetal receptor.

Coligan, Pardoll, Bluestone and associates[27–29] have demonstrated that at day 15 of gestation, only the γX proteins are associated with CD3 on the surface of thymocytes. By day 17.5, there are about equivalent amounts of γX and $\alpha\beta$ associated with thymic CD3; and by birth on day 20, no γX is detectable in unfractionated thymocytes.

On these data alone, the idea that the γ product is expressed early in ontogeny in precursors to the $\alpha\beta$-bearing cells is still tenable. But for several reasons it now seems much more likely that the γ-bearing cells comprise a separate T-cell lineage whose intrathymic development precedes that of cells expressing CD3–Ti $\alpha\beta$.

First, the γ protein on fetal and adult thymocytes bears *N*-linked carbohydrates and is therefore presumably derived from the $C_\gamma1$ gene segment which, unlike $C_\gamma2$, has sites for these glycans. This is significant because most mature $\alpha\beta$-bearing T cells contain only defective (out of frame) transcripts from the $C_\gamma2$ segment. Second, it has been possible to identify γ-bearing cells in the chicken, where the stages of fetal development are exceptionally well defined, and show that the precursor cells that migrate to the thymus seem to acquire Ti γX early in ontogeny but Ti $\alpha\beta$ later. Cooper and Chen (personal communication) have produced monoclonal antibodies against the chicken CD3, CD4 and CD8 structures as well as one against a receptor determinant (TCR1) that identifies a putative Ti γ epitope expressed on CD3$^+$CD4$^-$CD8$^-$ T-lineage cells in the chick and precipitates a disulphide-linked CD3-associated heterodimer with subunits of M_r 50K and 38K.

During fetal development precursor cells move into the thymic epithelium in discrete waves. The first wave, which is initially CD3$^-$, rapidly expresses CD3 and TCR1 but not CD4 or CD8. The CD3$^+$ TCR1$^+$ cells account for 30 % of thymocytes at embryonic day 15, but drop to less than 5 % by the day of hatching (day 21), while the frequency of CD3$^+$CD4$^+$-CD8$^+$TCR1$^-$ cells increases. Subsequent waves of thymic progenitors contain much smaller number of cells expressing TCR1, although they acquire CD3 and thus probably also Ti $\alpha\beta$ (as CD3 and Ti are believed to be obligatorily co-expressed). In the adult chicken, 10 % of thymocytes and 25 % of peripheral T cells react with TCR1. The data thus suggest that the CD3$^+$ Ti γ cells are a separate sub-lineage rather than a precursor of the CD3–Ti $\alpha\beta$-receptor expressing cells.

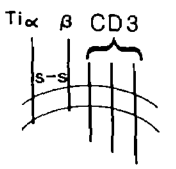
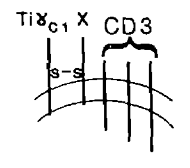
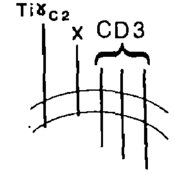
Receptor subunits			
Distribution	≥95% T cells	≤5% T cells	≤5% T cells
Phenotype	CD3+CD4+CD8– CD3+CD4–CD8+	CD3+CD4–CD–	CD3+CD4–CD8–
Ontogeny	later	earlier	earlier
Function	T_H, T_C, T_{NK}, ?T_S	NK-like, ?other	NK-like, ?other

Fig. 2 Phenotype and pattern of expression of T-cell receptors for antigen. The receptor dimers (Ti) are shown in association with the three CD3 subunits, CD3 γ, CD3 δ and CD3 ε, referred to collectively as CD3. The Ti γ partner, which has been termed δ by some, is denoted here as X to acknowledge the possibility that it represents an unknown C_γ product and to avoid confusion with the CD3 δ subunit (note that there is already scope for confusion between Ti γ and CD3 γ). Possible additional subunits are not included[34]. Also note that the less commonly identified CD3$^+$CD4$^-$CD8$^+$Ti γX phenotype is not shown. Other cell-surface molecules defining the phenotype are designated CD4 and CD8. Their function is not known for sure but they are strongly implicated in enhancing the binding of the T-cell receptor to MHC molecules. As a rule, CD4 performs this function on T helper cells (generally MHC class II-restricted) and CD8 on cytotoxic cells (generally MHC class I-restricted). Neither of these molecules is present on the surface of T-lineage cells early in thymic development, but it is believed that the precursors to mature functional T cells expressing the Ti $\alpha\beta$ receptor express either or both.

It is far from clear what regulates differentiation along CD3–Ti γX as against CD3–Ti $\alpha\beta$ pathways. However, CD3–Ti γX clones generally have one functional γ gene rearrangement and usually no β gene rearrangements whereas, in contrast, CD3–Ti $\alpha\beta$ clones have several non-functional γ gene rearrangements and functional β gene rearrangements, so that a stochastic model may be applicable. Thus, if γ gene rearrangement is productive, productive β and α gene rearrangement fails to occur. Alternatively, if γ gene rearrangement is unsuccessful in both alleles, rearrangement attempts continue. Nevertheless, such a model does not readily explain why CD3–Ti γX as opposed to CD3–Ti $\alpha\beta$ expression is mostly limited to thymocytes of the CD4$^-$CD8$^-$ phenotype.

Why have several forms of T-cell receptor? Certainly, CD3–Ti γX receptors lack the extensive germline diversity of their CD3–Ti $\alpha\beta$ counterparts, implying that the specificities they encode are more limited. Could this receptor complex have been the phylogenetic precursor of the $\alpha\beta$ heterodimer? If so, it is far from vestigial, given the precise maintenance of recombination-recognition signals in the various Ti γ gene segments and the nature of the γ gene protein.

There seem to be two other possibilities, not mutually exclusive. The CD3–Ti γX receptor that appears early in ontogeny may provide protective immunity to the fetus against common viral or other pathogens before the development of MHC-restricted CD3–Ti $\alpha\beta$ receptor structures. In the mature peripheral compartment, the CD3–Ti γX receptor may encode specificities not provided by the CD3–Ti $\alpha\beta$ repertoire. This would make it perhaps analogous to the minor light chains of immunoglobulins, which although less diverse than the major class nonetheless make a significant contribution to total antibody diversity. It is worth noting that the δ subunit of the CD3 complex associated with γX has a different electrophoretic mobility from its counterpart in the CD3–Ti $\alpha\beta$ receptor[1,2]. It remains to be determined whether this difference is due to glycan or peptide. But either could signify differences in the physiology of T-cell activation by the different receptors. Differences in refractoriness or sensitivity to triggering events may be important in functionally diverse and perhaps ontogenetically distinct populations, such as suppressor T lymphocytes.

Finally, there is the obvious outstanding question of the origin of the Ti γ-associated X chain. Is this also a structure with a variable domain encoded by a rearranging set of genes? Variability in peptides should be detectable by comparison of X chains from different clones, as it was in the earlier investigations on the Ti α and β subunits. Identification of X will elucidate whether Ti $\cdot\gamma$ associates only with X or whether there are still other chains yielding still more types of receptor. It is possible that the recently identified murine $C_\gamma4$ locus[28], which is only weakly homologous to the other C_γ loci and which contains *N*-linked glycosylation sites, may encode a Ti γ partner. ☐

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Photosynthesis

Structure of key enzyme refined

Jim Barber

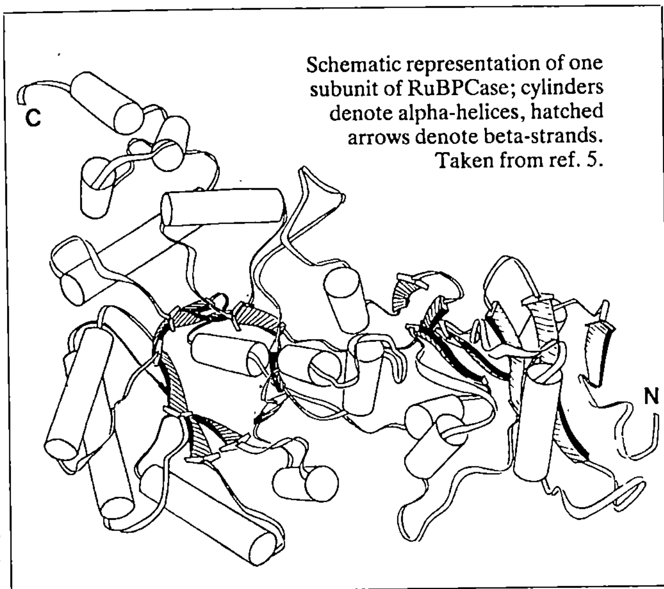
DETERMINATION of the structure of the leaf protein ribulose- 1,5-bisphosphate carboxylase/oxygenase (RuBPCase) is likely to be an important step for improving crop productivity by increasing the efficiency of photosynthesis. On page 730 of this issue[1] Holzenburg *et al.* identify the conformational changes that occur when substrate binds to the higher-plant form of this enzyme, an important result for future attempts to 'engineer' crop efficiency.

RuBPCase, the most abundant naturally occurring enzyme[2], initiates the reduction of atmospheric carbon dioxide to organic molecules during photosynthesis. Every molecule of fixed carbon in the biosphere originates from its catalytic activity, and it catalyses the fixation of about 10^{11} tons of CO_2 per year. Despite the key role of this enzyme, it does not work at maximum efficiency. One reason for this is that it catalyses both the carboxylation of ribulose-1,5-bisphosphate and its oxygenation[3]. The oxygenase activity results in photorespiration, which substantially reduces the overall rate of photosynthesis and consequently plant productivity.

It has frequently been argued, therefore, that it will be necessary to reduce or eliminate the oxygenase activity of RuBPCase to improve the efficiency of photosynthesis. Some plants, called C4 plants, overcome the carboxylase/oxygenase problem by increasing the relative level of CO_2 at the reactive site of the enzyme[4]. In general these plants are tropical or subtropical species, such as sugar cane. Most plants growing in temperate climates, however, are C3 type which use only the reductive pentose phosphate cycle to fix carbon and therefore support significant levels of photorespiration. At present, the only method for increasing their productivity is to grow these plants in an atmosphere enriched with CO_2, a valuable procedure in the horticulture industry, where greenhouses are used for cultivating commercially important crops. But for crops grown in the field, and therefore subjected to atmospheric concentrations of CO_2, the hope for increasing photosynthetic efficiency is to produce new varieties of plants containing RuBPCase with reduced amounts of oxygenase activity. Attempts to isolate mutants with this preferred characteristic have so far been unsuccessful and the only solution seems to be the application of *in vitro* genetic engineering using recombinant DNA techniques. To achieve this, however, will require a complete understanding of the catalytic mechanism of the enzyme in terms of its molecular structure.

RuBPCase in higher plants and in a range of other organisms, including algae and photosynthetic bacteria, consists of eight small and eight large subunits (L_8S_8). In higher plants and algae, the S subunits are encoded by the nucleus whereas the L subunits are encoded by the chloroplast genome. It is known that the catalytic site is located on the L subunits and that a specific lysine residue becomes carbamylated by an activator CO_2 molecule and stabilized by a magnesium ion. This activation process is necessary for both carboxylation and oxygenation reactions. The activated ternary complex can react with the substrate ribulose-1,5-

bisphosphate, which is subsequently attacked either by CO_2 or by O_2. All the biochemical evidence indicates that both carboxylation and oxygenation reactions occur at the same site in the protein.



Schematic representation of one subunit of RuBPCase; cylinders denote alpha-helices, hatched arrows denote beta-strands. Taken from ref. 5.

Significant recent advances have been made in the attempt to link the enzymology of RuBPCase with its structure. Schneider *et al.*[5] have successfully obtained the three-dimensional structure to a resolution of 2.9 Å of a simpler form of the enzyme, consisting of only two large subunits (L_2) and isolated from the photosynthetic bacterium *Rhodospirillum rubrum* (see figure). These authors showed that the active site is located in a carboxy-terminal domain which has an eight-stranded parallel alpha/beta-barrel structure. The three lysine residues in the site are in loops at the carboxy ends of the beta-strands, regions which have very similar amino-acid sequences to the enzyme in higher plants. Although the L_8S_8 form of the enzyme in higher plants was crystallized several years ago[6], its large relative molecular mass (550,000) and its complex structure have hampered analyses of data obtained by X-ray diffraction.

Various techniques indicate that the L_8S_8 complex has a diameter of 124 Å and a height of 100 Å, and Holzenburg and colleagues in this issue[1] now present the structure of the L_8S_8 form in more detail. They isolated the enzyme from the hydrogen-oxidizing bacterium *Alcaligenes eutrophus* and crystallized it in its activated state with and without the intermediate substrate 2-carboxyarabinitol-1,5-bisphosphate (CABP). The authors used X-ray crystallography and electron microscopy to obtain structural information to a resolution of 5 and 17 Å, respectively. Both methods give comparable results and show that binding of the substrate results in a new structural state of the enzyme. The L_8S_8 complex consists of two L_4S_4 halves, each with 4-fold symmetry. After substrate binding, the 4-fold axes of the L_4S_4 halves no longer coincide, being shifted by 36 Å and related by a 2-fold axis perpendicular to, and between, the local 4-fold axes. The detection of this conformational change after substrate binding supports earlier X-ray work[6] with the tobacco enzyme and biochemical studies[7] with the spinach enzyme.

Unfortunately, the spatial resolution of the structure described by Holzenburg *et al.* is not sufficient to begin engineering the enzymic properties of RuBPCase. To pinpoint the exact positioning of potentially important amino acids within the protein it is necessary to know the organization of the L_8S_8 complex at a resolution of 3 Å or less. As mentioned above, the structure of the L_2 enzyme of *R. rubrum* has just been solved at this level of resolution[5], and indeed its active site has already been modified by site-directed mutagenesis[8-11]. It seems most likely, therefore, that for the time being work on the simpler bacterial L_2 form will lay the foundations for establishing whether it is possible to increase the carboxylation reaction of the enzyme while reducing its oxygenic activity.

The work of Holzenburg *et al.* does, however, have important implications regarding the diurnal variation of RuBPCase activity, which seems to be under the control of an inhibitor molecule very similar to the CABP substrate used in their structural studies. The identity of this natural reversible inhibitor was recently reported in *Nature*[12] by Gutteridge and colleagues, who showed it to be 2-carboxyarabinitol-1-phosphate. □

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Solid-state physics

Superconductivity at high temperatures in doped oxides

M. Strongin, D.O. Welch and J.W. Davenport

High-temperature superconductivity has always been something of an oxymoron. Although there have never been any theoretical prohibitions against superconductivity at, for example, room temperature, it has always been a distinctly low-temperature affair: until very recently, superconductivity has been confined to temperatures less than 23 K, notwithstanding considerable theoretical effort exploring various exotic mechanisms and several experimental false alarms. But high-temperature superconductivity is a phenomenon of enormous importance and potential as it holds the possibility of resistanceless transmission of electrical power and the possibility of powerful electromagnets and generators that would operate without electrical resistance. Nonetheless, progress in raising the temperature T_c of the transitions to the superconducting state has, until now, been frustratingly slow, and in recent years the search for new high-T_c materials has lost impetus. The recent discovery of oxide superconductors with T_c values greater than 30 K has brought new enthusiasm to superconductivity research.

The apparent barrier to T_c above 23 K has been lifted by the discovery of the $La_{2-x}X_xCuO_4$ superconductors (where X is, for example, Ba, Sr or Ca), and it seems reasonable that superconductivity in other similar compounds will be discovered; furthermore the real possibility exists for superconductivity at liquid-nitrogen temperatures and higher. The recent meeting on 20 January at the Argonne National Laboratory was held to exchange information and to coordinate the already active programmes in Department of Energy laboratories in the United States, and some of the experiments we discuss here were reported at the meeting. The technological ramifications are obvious for power transmission, magnets, superconducting electronics and other electrical devices, and there are already speculations about new magnets for planned accelerators. This is probably somewhat premature because of the length of time usually necessary to go from samples in the laboratory to the long lengths of wire necessary to wind magnets. But it is clear that this is only a matter of time, and if the fuzzy reports of onsets near 70 K described below turn out to be true superconductivity, then electrical technology may truly be revolutionized in a few years.

The phenomenon of superconductivity was discovered in 1911 by Kammerlingh Onnes, who found that mercury became superconducting at about 4.2 K. In 1954, superconductivity in the A-15 compounds was discovered, with a T_c of 17.1 K in V_3Si, quickly followed by the discovery of Nb_3Sn with a T_c of 18.6 K. It then took 20 years until Nb_3Ge was discovered, and this compound set the previous limit for T_c of about 23 K. Until a few months ago there had been no substantial progress in raising T_c, notwithstanding a few false alarms, most notably the case of CuCl. Most of the researchers in this area had begun to have a mystical or cynical view that superconductivity above about 23 K was impossible, and there were various speculations about the instabilities limiting the highest possible T_c.

Given this history, it is probably not surprising that when the great event happened it was accepted rather gradually. The predecessor of the exciting new results was the discovery[1] of superconductivity in the mixed-valent perovskite-structure oxide $BaPb_{1-x}Bi_xO_3$ with a T_c of 13 K. Then Bednorz and Müller of IBM Zurich Research Laboratory found evidence[2] of a much higher T_c in a multiphased sample in the Ba–La–Cu–O system. Even with this evidence of superconductivity at 'high' temperatures, most researchers did not get very excited, possibly because Bednorz and Müller did not strongly associate their resistive anomalies with true bulk superconductivity, and suggested that the high onset temperature was caused by two-dimensional fluctuations. Probably because of false alarms and unconfirmed reports of high-temperature superconductivity in other exotic systems, most workers in the field appeared wary. It seems that the work of Uchida *et al.*[3] at the University of Tokyo, which both confirms and extends the work of Bednorz and Müller, and shows higher-quality samples with real transitions, finally found general acceptance when it was presented at the Materials Research Society meeting in Boston, 1–6 December.

Uchida *et al.* made the important step of showing that the high-T_c phase in the multiphased material of Bednorz and Müller was $La_{2-x}Ba_xCuO_{4-y}$ with $x \sim 0.1$, which has the layered perovskite K_2NiF_4 structure. This crystal structure can be viewed as a stacking of LaO–CuO_2–LaO sandwiches. It has been suggested that the states responsible for the superconductivity probably lie in the central CuO_2 plane. These states should be essentially two dimensional because the distance between CuO_2 planes is quite large (more than 6 Å). Whether this two dimensionality is crucial to superconductivity is not known, but in recent years there has been some interest in low-dimensional systems such as the organic superconductors, which in some ways are one dimensional.

Since the beginning of December 1986 there have been rapid developments, which have been announced in several newspaper accounts and press conferences. C.W. Chu and co-workers at the University of Houston have reported onset of superconduction near 70 K and sharp regions in the transition as high as 60 K, and they show[4] that the transition in La–Ba–Cu–oxide can be enhanced by pressure. R.S. Cava *et al.* at AT&T Bell Laboratories, on the other hand, have substituted Sr for Ba and obtain[5] sharp transitions as high as 38 K. In the meantime, workers in China (Z. Zhao *et al.*, Chinese Academy of Sciences preprint) have reported onsets near 50 K and full transitions above 40 K in both Sr- and Ba-doped $LaCuO_5$ with some evidence of onsets near 70 K. Similar results have been obtained in several other laboratories (for example, ref. 6).

One exciting prospect of such a large increase in the record value of T_c is that it could be the consequence of an exotic new mechanism, such as excitonic or bipolaronic mechanisms, for the interactions between electrons which leads to the formation of Cooper pairs, and hence superconductivity. But, the values of various characteristic superconducting parameters obtained so far for the new material indicate that although the parameters might be somewhat unusual, the superconductivity is caused by the usual electron–phonon interaction mechanism. Three of the parameters are the density of electronic states at the Fermi level, the electron–phonon coupling strength and the frequency of the interacting phonon modes. The density of states appears to be lower than in previous high-temperature superconductors, such as Nb_3Sn, but apparently the low density of states is more than compensated for by the large electron–phonon coupling.

As well as the vast array of conventional measurements in progress on the magnetic and electrical properties of these superconductors, microscopic probes are being used to elucidate geometrical and electronic structure of the superconducting phase. The aim of this work is to try to understand the fundamental mechanism for the superconductivity and to test the theoretical ideas that are already appearing, for example, the possible quasi-two-dimensional nature of the relevant electron bands and the role of a concomitant Peierls transition. Inelastic neutron-scattering experiments are already planned that will address the question of whether 'soft-phonon' modes dominate the electron–phonon coupling, and this work should be well under way by the time this article is published. X-ra experiments at the new ge synchrotrons are also active, reveal structural instabilities i plex crystal structures. The should help to explain how th ductivity is related to the struc er X-ray absorption experime bing the CuK edge to examine state of the copper in the o experiments are even using probe the magnetic-field distri have already shown how th field falls off into these materi

The sceptics may questio these compounds truly exhib ductivity, or just show a tran one metallic state to a metallic three or four orders of magn conducting. In other words, is superconductor with zero elec ance? Although the initiall work has not yet addressed thi Bell group has measured[5] th effect in these samples, an Bednorz and Müller[6]. To the a this is a true indication of supe ity, as it implies that the san magnetic flux when cooled in field. A highly conducting me do this, only a true supe Although persistent currents a sistance have not yet been dem is almost certain that this is tru ductivity and that persistent cur

The future for the new hi superconductors appears bri assessment of their potential requires the accurate deter some crucial parameters. For e be useful in high-field magnet erial must remain supercondu magnetic fields while car amounts of electric current. values of the critical currents (above which the sample ret normal state) still appear s almost certain that a large p results from the sample conditi perfections. Even if the criti turns out to be intrinsically sma not preclude a very useful ro conducting electronics. Anot tant parameter is the critica field (the magnetic field above sample returns to the normal although there is huge variat initial estimates, it seems that field at 0 K can be greater th (compared with ~ 30 tesla for N

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X-ray astronomy

Observations of active galaxies

Richard Mushotzky

WITH the launch of the EXOSAT satellite in 1983 it became possible to look at the X-ray variability of active galactic nuclei in detail[1]. The sensitivity and unprecedented capability of EXOSAT to perform continuous observations of up to 80 hours made it possible for the first time to characterize carefully the nature of the variability in a small but significant sample of 'normal' active galactic nuclei. Extremely high-quality observations are available for timescales from minutes to a day for more than ten active galactic nuclei and for timescales of up to 6 hours for many more; of the ten, at least six seem to show interesting variability. The first detailed analysis of these long looks is described in two papers on pages 694 and 696 of this issue[2].

One of the goals of the study of active galactic nuclei is the determination of fundamental properties such as mass, accretion rate and physical processes responsible for the emission. Simple theoretical arguments suggest[4] that these qualities can be derived from study of the X-ray variability. The fastest repeatable timescales should be related to the radius at which most of the energy of liberated. If the active galactic nuclei were accreting massive black holes, this would be of the order of 5 Schwarzschild radii[5]. The corresponding timescale is $\sim 50\,M_6$ seconds, where M_6 is the mass of the central black hole in units of 10^6 solar masses. Longer characteristic times could be caused by heating/cooling of the radiating plasma or acceleration timescales for the particles.

A possible analogy between active galactic nuclei and the best-studied galactic black-hole candidate Cyg X-1 is intriguing. The X-ray light curve of Cyg X-1 is chaotic and often shows large amplitude variability. It appears to have two characteristic timescales, one of the order of milliseconds[6], which is of the right order of magnitude for the 'observed' 10-solar mass black hole; and the other of the considerably longer timescale[7] of about 1 second, which has never been convincingly explained.

Before the launch of EXOSAT, observations of X-ray time variability of active galactic nuclei could be understood as distinct flares superimposed on more-or-less consistent sources. These flares seemed to have well-determined doubling times; the best case was established for the very bright Seyfert 1 galaxy NGC4151 (refs 8,9). On shorter timescales most objects appeared not to show frequent large-amplitude variability[10]. The absence of data gaps and the quality of the data provided by EXOSAT allows detailed investigation of the form of the temporal variation of active galactic nuclei in a manner similar to studies of the galactic black-hole candidates Cyg X-1, GX339-4 and others[2,3,11].

The new EXOSAT results[2,3] add considerable complexity and differ in two significant ways. First, many more objects are seen to vary on short timescales[1]; and second, in some cases the character of the time variability is quantitatively and qualitatively different than expected from the flare 'model'. This is best seen in the power-density spectra of the variability in the two active galaxies NGC5506 and NGC4051 reported in this issue[2,3]. These spectra are relatively featureless power laws of more than one decade in frequency f with a $\sim f^{-1}$ power spectrum. Such a spectrum is similar to that of turbulence and arises in many physical circumstances, but is poorly understood. Because the $\sim f^{-1}$ character of the power-density spectra persists over the frequency range sampled, the quantity $F/(\mathrm{d}F/\mathrm{d}t)$, where F is source flux, is not uniquely defined, and "there are no preferred timescales"[2,3]. In the case of a power-law power-density spectrum, the 'variability timescale' is model dependent and is a function of the signal-to-noise ratio and of observation length[3]. Thus, these results cast doubts on previous interpretations of the observed time variability in active galaxies[12], which indicated that there is a fundamental and simple relation between source luminosity and variability time. To confuse the situation further, another active galaxy, NGC4593, may not have a pure power-law spectrum[1] and autocorrelation analysis of this object gives sensible timescales which vary as the luminosity of the object, agreeing with the previously published correlation[12].

Previous reports[13] on the time variability of NGC4051 used the autocorrelation function to demonstrate that this object had a characteristic variability timescale. Lawrence *et al.*[2] argue that as the power-density spectrum is the Fourier transform of the autocorrelation function, it must carry the same information and that there are problems in the interpretation of the function in the presence of low-frequency divergent noise. Of course, the spectrum cannot diverge to arbitrarily low frequencies because active galaxies do not in general show high-amplitude flux variability on long timescales[14,15]. In particular the three objects I discuss here do not. The present two-day observations of NGC4051 and NGC5506 sample the entire known range in variability amplitude. This suggests, at least for these galaxies, that the characteristic variability timescale, the 'knee' in the power-density spectrum, is constrained to be less than a few days and no longer than for a few hours. Scaling of the observed knee in Cyg X-1 by the ratio of the luminosities would give a 'predicted' turnover in the power-density spectrum for NGC4051 of 5×10^{-6} Hz, which is just below the observational range from EXOSAT[2]. It is thus conceivable that the sought-for variability timescale is just longer than the low-frequency observational limit of EXOSAT.

The faintness of the sources and the relatively small size of the EXOSAT detectors do not yield sufficiently sensitive data to study the high-frequency ($> 10^{-3}$ Hz) regime. Models of Eddington-limited luminous active galaxies predict a frequency that is an order of magnitude higher than for the characteristic time of material near a few Schwarzschild radii. The Japanese X-ray astronomy satellite ASTRO-C, which will be launched this month, may have the sensitivity necessary to sample this high-frequency domain. The most interesting observations will be those that determine the frequency at which the sources cease to vary.

100 years ago

THIS is one of the most interesting books of travel that it has been our good fortune to meet with for several years ["The cruise of the Marchesa to Kamschatka and New Guinea with notices of Formosa, Liu-Kiu and various islands of the Malay Archipelago" by F.H.H. Guillemard]. "Nearest us was Tolbatchinska; and beyond rose the twin peaks of Kojerevska and Kluchefskaya, pyramids of the purest snow, before which one felt how poor was all language to express the sense of their perfect beauty.

From *Nature* **35**, 369; 17 February 1887.

This should be characteristic of the shortest length scales or the fastest physical processes.

To determine if the 'true characteristic' timescales are as long as suggested by the scaling of the 1-second time variability from Cyg X-1, long observations, of roughly 10 days' duration, will be required. As for Cyg X-1, this long timescale is not directly relevant to black-hole models for objects accreting at the Eddington limit. It has been suggested[12], however, that low-luminosity active galaxies are emitting at $\sim 10^{-3}$ of the Eddington limit; if so, these long timescales are exactly as predicted.

The EXOSAT results have improved X-ray timing observations of active galaxies to the point where realistic detailed models are necessary to interpret the data. In some ways this situation is similar to that of brownian motion before its explanation by Einstein. We now have high-quality time variability data with no obvious signatures. It is to be hoped that the ASTRO-C observations will greatly increase the database and determine whether X-ray timing measurements can indeed constrain black-hole models of active galaxies. □

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Analytical geochemistry

Surfaces of reacted minerals

Harold C. Helgeson

UNTIL recently, much of what we know, or think we know, about the mechanisms of interfacial reactions between silicates and aqueous solutions has been derived from subjective interpretation of indirect (and in many cases ambiguous) evidence, largely rate studies of mineral hydrolysis. Now, however, advanced technology is being used to examine and to analyse directly the surfaces of reacted mineral grains to determine the composition of their surface 'skins' and the extent to which they change with reaction progress. These data can be used to infer the stoichiometry of activated complexes at reactive surface sites and to elucidate the mechanisms of surface detachment during the dissolution of complex minerals such as feldspars, pyroxenes and amphiboles. Recent pioneering studies of this kind, one of which is reported by Jean-Claude Petit *et al.* on page 705 of this issue, use the specific resonant nuclear reaction technique to measure the total hydrogen concentration (H^+, H_2O and H_3O^+) as a function of depth to about 1,000 Å from the outer surface of reacted diopside ($CaMg(SiO_3)_2$) grains at *p*H values of 2 and 6. The results of Petit *et al.* indicate that one or more of these species is present in high concentrations at depths of about 500 Å from the outer surface of the reacted diopside grains, and that the concentration is maximal at a depth of about 200 Å. Circumstantial evidence indicates that most of the hydrogen is present as H_2O, but note that small concentrations of H_3O^+ relative to H_2O in the hydrogenated surface are sufficient to control the rate of mineral hydrolysis.

The steady-state *p*H dependence of mineral dissolution rates observed in the laboratory can be explained in terms of transition-state theory by assuming the presence of activated complexes involving hydrogen, hydronium or hydroxyl ions at the reactive sites. The stoichiometry of these activated complexes depends on the solution *p*H and the crystallochemical characteristics of the mineral. For example, recent theoretical consideration by W. M. Murphy (*Geochim. cosmochim. Acta,* in the press) of pyroxene, wollastonite and olivine dissolution rates reported in the literature indicates that the dissolution stoichiometries and *p*H dependence of these rates are consistent with steady-state rate control by transition-state decomposition of activated complexes with stoichiometries corresponding to $(H,C(2))_{0.5}C(1)_{0.5}SiO_3^Z$, $(H_2Ca)SiO_3$ and $H(Mg,Fe)_2SiO_4^+$, respectively, where *Z* is the charge on the complex and C(1) and C(2) are the cations on the M(1) and M(2) octahedral sites, respectively. The resonant nuclear reaction technique and secondary ion mass spectrometry offer hope of direct confirmation of the existence of these species at surface reactive sites such as dislocation outcrops. But much remains to be done to discriminate between H^+, H_3O^+, H_2O, OH^- and other species at particular sites. At the moment, only the total concentrations of elements can be measured, and even these are averaged over the surface of the mineral.

In addition to these two techniques, X-ray photoelectron spectroscopy is a powerful method of analysing the surfaces of reactant minerals. This technique has been used to demonstrate that Ca and Mg in the M(2) octahedral sites of dissolving diopside are preferentially released relative to SiO_2 from the outermost surface of the reacting mineral (Schott, J., Berner, R. A. & Sjöberg, E. L. *Geochim. cosmochim. Acta* **45**, 2123–2135; 1981, and Schott, J. & Berner, R. A. *Geochim. cosmochim. Acta* **47**, 2333–2340; 1983). Comparison of results using all three techniques indicates that the relation between penetration of hydrogen into the surface during dissolution and remobilization of the elements of the reactant mineral at the active surface sites is far more complex for crystalline silicates than for simple silicate glasses.

Studies of reacted mineral surfaces using the three methods I have mentioned above have important implications for many geochemical processes, including weathering, diagenesis and hydrothermal rock alteration. In addition, they should allow better understanding of reactions among ground waters and toxic waste materials, such as solid radioactive waste. Experimental techniques of this kind will help to elucidate the surface chemistry of silicates and hold great promise for unravelling the mechanisms of heterogeneous catalysis on mineral surfaces of both organic and inorganic reactions in geochemical processes. These include decarboxylation reactions and reduction of sulphate in natural waters.

Reactions among mineral surfaces and aqueous solutions is a complex process involving such phenomena as hydration of the surface, adsorption, ion exchange, complex formation, bond disruption and irreversible decomposition of activated complexes followed by surface detachment. Depending on the stoichiometric number per mole of energetically distinct crystallographic sites for cations present in a mineral, which ranges from three in pyroxenes such as diopside to nine in certain amphiboles, many surface species may be involved in the hydrolytic process. In particularly complex minerals such as amphiboles, dissolution mechanisms may involve parallel reactions rather than a simple series of elementary reaction steps. Only when technology is sophisticated enough to detect and unravel these complex mechanisms will we be able to claim with confidence that we can hope to understand the hydrolytic process. □

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Vagaries of nomenclature

SIR—It is a pity that when zoological nomenclature is an Aunt Sally for non-taxonomists they should so often be uninformed or disingenuous. Seldom do they trouble to find a reason for a nomenclature requirement that seems to them arcane or futile. So it is with L.J. Bruce-Chwatt's remonstrance (*Nature* **325**, 114; 1987) against the use of '*-i*' and '*-ii*' terminations for patronymic names of mosquito species. He argues that the '*-ii*' ending is 'wrong', because names such as *bancroftii* and *cruzii* honour Bancroft and Cruz, not Bancroftius and Cruzius, and therefore that by his lopping of the terminal '*-i*' when there are two he is using the 'grammatically correct spelling'.

This would be fine were it not for the fact that notional latinization of modern personal names (to Bancroftius, for example) before forming the genitives has been widespread and legitimate practice since the dawn of Latin-based nomenclature and is still permissible today. Would he have us 'correct' the multitude of such '*-ii*' names published since 1758 in every major animal group — or is he really after a dispensation for malariologists? Anyway, he should take heart. The use of '*-ii*' personal genitives for new species has been effectively dead for many years and is recommended against in the International Code of Zoological Nomenclature (Appendix D(III)16(b)).

Bruce-Chwatt fails to note the obvious risk inherent in what he has done, namely that those without his classical grounding (most of us), seeing that he reduces '*-ii*' to '*-i*', might assume that the second 'i' should always be lopped. He does not mention the point, but the names of several mosquitoes correctly end '*-ii*' because they are genitives based on personal names (usually Italian, Indian or Japanese) that happen to end with the letter '*i*'; *ficalbii, forattinii, goeldii, hatorii, martinii, onorii, purii*, are examples. There is also *fabricii* from the great (latinized) naturalist Fabricius and the geographically derived *mississippii*.

Under the present code, the correct spelling is the one published when the species was first described, whether or not the '*-i*' or '*-ii*' termination was used. This allows the correct spelling to be objectively determined by reference to that description — and obviates the need to trace all the subsequent history of the name in its scattered literature in an effort to discover who first altered it (or indeed whether it has ever been altered at all). Under the code all is clear; a variant subsequent spelling (one '*i*' reduced to two or vice versa) is an 'incorrect subsequent spelling' (Article 33(d)) and cannot be validly used. The much-maligned and misunderstood code is right on this particular issue.

I cannot refrain from noting the paradox with which Bruce-Chwatt ends his letter. He blames the code for preserving stability, an unsettling experience for taxonomists used to obloquy for supposedly doing the opposite.

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Divergence and directional mutation pressures

SIR—Grivell[1] proposes that divergent genetic codes, as found in ciliated protozoa and *Mycoplasma* may be "relics of a primitive genetic code [that] possibly preceded the emergence of the so-called universal code". We suggest that a different explanation is more probable, and that such codes may be formed by capture of stop codons. Clues to this event in the case of ciliates are found by comparing the sequences of transfer RNAs.

Kuchino, Hanyu and co-workers[2,3] found two 'new' glutamine tRNAs in *Tetrahymena thermophilus*, one with the anticodon UmUA (where Um is 2′-*O*- methyluridine), pairing with UAA and UAG, and the other with anticodon CUA, pairing with UAG. UAA and UAG are stop codons in the universal code, but in ciliated protozoa the only stop codon appears to be UGA. Differences in nucleotide sequences between the 'new' tRNAs and the other glutamine tRNA, with anticodon UmUG, in *Tetrahymena*, are 13 and 11 nucleotide substitutions in $\mathrm{tRNA}^{\mathrm{Gln}}_{\mathrm{UmUG}}$ when compared with $\mathrm{tRNA}^{\mathrm{Gln}}_{\mathrm{UmUA}}$ and $\mathrm{tRNA}^{\mathrm{Gln}}_{\mathrm{CUA}}$ respectively, and 4 substitutions in $\mathrm{tRNA}^{\mathrm{Gln}}_{\mathrm{UmUA}}$ compared with $\mathrm{tRNA}^{\mathrm{Gln}}_{\mathrm{CUA}}$ (ref. 3). These differences may be a rough clue as to time elapsed since evolutionary divergence[4]. Differences between yeast and rat or wheat tRNAs with the same anticodon are 13–15 substitutions[5] and between *Drosophila* and vertebrates are 5–12 substitutions[5]. From this, one could conclude that the divergences of the tRNAs for Gln in *Tetrahymena* were well within the time of existence of eukaryotes.

Capture of stop codons could have taken place through a series of non-deleterious (neutral or adaptive) changes brought about by 'directional mutational pressure'[6]. Stop codons UAA and UAG have evidently disappeared from ciliates, perhaps during successive episodes of AT and GC pressure. AT pressure presumably now exists in ciliates, as shown by the low GC content (20–30 per cent) of their DNA. The gene for $\mathrm{tRNA}^{\mathrm{Gln}}_{\mathrm{UmUG}}$ duplicated and one of the duplicates, under AT pressure, acquired anticodon UmUA. Later, the tRNA gene with anticodon UmUA duplicated again, and in one of the duplicates, anticodon UmUA mutated to CUA, which pairs strongly with UAG[3].

During this period, some of the glutamine codons CAA and CAG mutated under AT pressure to UAA and UAG, pairing with the new glutamine anticodons UmUA and CUA. UAA and UAG have been identified as alternate glutamine codons in the ciliated protozoans *Tetrahymena*[7], *Stylonichia*[8] and *Paramecium*[9,10]. This may be a result of recent stop codon capture[11]. This is in contrast to the suggestion by Grivell[1] that "the ciliates branched off from the primitive eukaryotic ancestor very early in evolution, quite possibly at a time when protein translation was still tolerant of changes in the genetic code". No such tolerances need be postulated, for the observed changes are compatible with neutral substitutions, and account for the presence in *Tetrahymena*, of four glutamine codons, CAA, CAG, UAA and UAG, and three glutamine anticodons, UUG, UUA and CUA. An analogous pattern exists for leucine in the universal genetic code with codons CUA, CUG, UUA and UUG and anticodons, UAG, UAA and CAA.

Similarly, in *Mycoplasma capricolum* (25 per cent GC in DNA) AT pressure has resulted in the disappearance of UGA stop codons, replaced by UAA, and the mutation of anticodon CCA, in a duplicate of tryptophan tRNA, to UCA[12]. Simultaneously, tryptophan codons UGG mutated to UGA. Anticodon UCA pairs with both UGG and UGA by 'wobble'. UGA has become a tryptophan codon, 'captured' by AT pressure[12,13].

We propose that *Tetrahymena* and *Mycoplasma* have both evolved to make translatable use of former stop codons[14] and that the divergent code in ciliates may be of recent origin and derived from the eukaryotic code.

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The pure and the applied

Robert L. Sinsheimer

Biotechnology: The University– Industrial Complex. By Martin Kenney.
Yale University Press:1986. Pp.306. $23.95, £22.50.

IN UNDER a decade, a whole new industry, capitalized with many billions of dollars, has come into being. Such is the rate of change in the late twentieth century. The product of 30 years of government support of basic biomedical research, biotechnology emerged from its incubator in the mid-1970s and quickly found its niche in the socio-economic structure of American and multi-national industry.

Mr Kenney chronicles, in intimate detail, the unfolding patterns of this development as determined by the extraordinary constraint that the basic knowledge — the blueprint for the industry — was almost exclusively to be found in the minds of university faculty. Industry had the production facilities and the marketing skills, but it was barren of the new expertise. The professorial start-up companies, the university–industry contracts, the limited partnerships in research and development, the equity purchases and licensing agreements by the multinational corporations, all stemmed from this unprecedented circumstance.

Mr Kenney's book is repetitious and much of the material is redundant, but it does draw together a great deal of information about the establishment, financing and management of the new biotechnology companies. Also described are the subsequent contractual and fiscal interventions of the large chemical and pharmaceutical companies, as well as their successful efforts to purchase, in whole or in part, access to or even sole rights to the output of appropriate university departments, so as to acquire instant expertise and to ensure the economic future of the companies concerned.

Particular attention is paid to agricultural biotechnology, potentially the largest market. Organizational arrangements here were complicated because the land-grant universities, traditionally the source of agricultural research, have not been in the forefront of molecular biology, which is largely funded instead by the National Institutes of Health. Other universities, such as Harvard, MIT, Washington (St Louis) and Stanford, have consequently become the corporate partners for the development of plant molecular biology.

The effects on the academic world of these university–corporate relationships are examined, perhaps necessarily, in less satisfactory detail. Only sketchy anecdotal evidence is presented as to the impact of corporate ties and proprietary information upon freedom of information in academia, upon the research and educational agendas, and upon the credibility of the universities as sources of disinterested judgement for society. In this regard, the author comments succinctly: "It is quite remarkable how quickly doubts regarding safety receded once it appeared that profits could be made from this new technology". Similarly, Mr Kenney makes only limited reference to the questions of the propriety of the sequestration to private gain of knowledge gained at public expense, or the longer term consequences of "validating new areas of the natural world as private property" via the patent system.

Genentech makes splash on Wall Street

"One of the most spectacular market debuts in recent history." That was how the *Wall Street Journal* described the first day of public trading in shares in Genentech, the San Francisco company which has been among the leaders of those aggressively pursuing the commercial exploitation of recombinant DNA technology.

Initially the company had proposed to offer one million shares at between $20 and $30 each. But demand was so great that an initial price of $35 was fixed for members of an underwriting syndicate through which the shares were made public last week. An extra 100,000 shares were made available, providing the company with an investment — once brokerage commissions had been deducted — of $36 million.

During the day of frantic over-the-counter trading, in which more than half of the shares were resold by their original purchasers, the price rose at one point to $89 a share, eventually dropping back to $71. As the company has 7.5 million shares this

Booming beginnings — a news story from Nature, October 1980.

The book is written from a capitalist–economic perspective, and does not cast a flattering light upon the molecular biology research community. Thus, on p.245 we find:

> The biomedical funding system created an entire research structure based upon grantsmanship and the building of research empires... . For the empires to keep functioning, products in the form of journal articles and prizes awarded are crucial. In this bitter competition research monies from any source are welcome because they could provide that competitive edge.

Mr Kenney is understanding, if not forgiving:

> The professors who join companies have made rational choices, given the alternatives presented... . Professors are also creatures of their social milieu. [This] is not to excuse the more egregious exploitation of students, the betrayal of the public trust, and even the theft that have occurred with creation of this industry.

He does not, however, relate these concerns to the responsibilities of universities to foster technology transfer — the transmission of the knowledge they have acquired with the aid of public funds to the industries wherein it may find useful application. Nor does he relate them to the increasing reliance, particularly in the United States, upon such high technology as the best means to maintain international competitiveness (which, in turn, affects the potential for university support). Such questions are particularly relevant to an industry by which, as the author states, "every production process that uses carbon-based materials" will be affected.

With the discovery that a previously arcane area of academic research suddenly had great market value, the traditional social relationships of the university became strained. Much of the recent development described here can be seen as an adaptation to that strain, but whether or not this is a transient adaptation remains to be seen.

It is only at the end of his book that Mr Kenney raises, suddenly and without answer, the most critical questions:

> •The entire manner in which man conceptualizes interaction with the natural world will change as we begin to create new life forms — making "natural" selection a planned rather than a random process. Our world will be filled with genetically engineered organisms: if DNA is a "program" then these are "living robots". Ultimately we will need institutions to deal with the fact that we can engineer ourselves [p.246].
>
> •Should the enormous power of transforming . . . life forms be transferred to groups merely seeking a return on investment [p.247]?
>
> •To be fully serious, the debate must ask the questions of what can and cannot be privatized and done for profit. Industry cannot answer this question — the managers have a duty to maximize profit... . This requirement for an unbiased debate raises the question of whether the universities and their professors are capable of carrying on this debate [p.246].

Biotechnology: The University–Industrial Complex bears upon questions of fundamental importance to science, academia and society, and provides valuable documentation of the magnitude of the actions already taken and the multitude of participants involved. But we must hope that someone will write the sequel, for which this is only the prologue. ☐

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What's going on in mathematics?

Thomas Banchoff

The Problems of Mathematics. By Ian Stewart. *Oxford University Press:1987. Pp.257. Hbk £17.50, $24.95; pbk £5.95, $9.95.*

FRACTALS, catastrophes and instantons — these are some of the strange objects that inhabit the current world of mathematics. We might see them once in a while mentioned in a newspaper article, quickly forgotten. We also hear that there are more mathematicians alive now than in all previous history combined. What is it that they all work on? What is the character of modern mathematical research and what is its relation to the familiar mathematics which is taught in schools? Is there any way for the lay person to begin to appreciate the answers to these questions?

Ian Stewart asks himself these things in an imaginary radio interview in the introduction to *The Problems of Mathematics*, and he concludes "Well, I suppose I could give it a *try*". He does in fact give it quite a good try. In 20 chapters, Dr Stewart surveys a great many of the areas of current research in mathematics, a formidable task if one accepts the assertion that most practising mathematicians have difficulty communicating their ideas to mathematicians outside their field let alone the lay person.

As a mathematican myself, I appreciated reading introductions to several areas I had only vaguely heard about, especially since the author has done well in presenting the subjects I know best. As a geometer, however, I have to remark on the paucity of visual material in the book. In all there are only nine illustrations (one of which, the cycloid curve traced out by a point on a rolling wheel, is in error in two respects) and fourteen chapters have no pictures at all. I find it almost impossible to imagine discussion of the mathematics of knots which contains not a single picture of one, or a treatment of non-Euclidean geometry without even one triangle. On the other hand, there is also an absence of equations and complicated algebraic symbolism. To tell so much about modern developments using neither equations nor extensive diagrams is quite an accomplishment.

The chapters are generally independent, which makes for easier browsing, and Dr Stewart starts most of them with a discussion of a classical problem which has some present-day aspect. From Euclid's proof of the infinitude of the number of primes, we move to factorization problems used in 'unbreakable' codes. From the discovery of non-Euclidean geometries in the last century, we come to modern research on possible geometries of three-dimensional analogues of surfaces. From elementary probability, we progress to the mathematics of chaos. The historical context is handled quite well, except that the problems of 20 or 30 years ago are somewhat neglected compared to those of the present day. Thus the research work of each of the Fields medallists (the mathematical equivalent of Nobel laureates) of the past decade is discussed but there is little mention of those who went before. For those who wish to fill in the intervening background, Dr Stewart provides a reading list for each chapter at the end of the book.

This is an unusually fine collection of essays about modern research mathematics. The other volumes in Oxford's *The Problems of . . .* series claim to be directed towards sixth-formers or first-year university students. Although these might certainly profit from reading Dr Stewart's book, it is definitely suited to a much wider audience. Indeed, it should perhaps be required reading for professional mathematicians as well, so that we might all benefit from the author's success in interpreting our work to the larger world. ☐

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One knock for "no"

Steve Blinkhorn

The Adventures of a Parapsychologist. By Susan Blackmore. *Prometheus:1986. Pp.249. $19.95.*

PARAPSYCHOLOGISTS, like Roman Catholics, come in two principal varieties: the devout and the lapsed. Whilst the memoirs of the devout are at best improving and uplifting, those of the lapsed can have all the morbid fascination of the autobiography of a public hangman.

Dr Blackmore, however, her life's allotted span barely half complete, has devised a new genre. Here one can read about the opinions, adventures, paranormal experiences and half-hearted extramarital amour (in the singular) of a failed parapsychologist. Too slight to be a treatise, uncertain as a melodrama and written in the style of pulp romantic fiction, it is an account of a change of faith.

Experiencing her self out of her body, the author seeks to work for a PhD in extra-sensory perception (ESP), and is told she is out of her mind. With growing methodological rigour, she consistently fails to find any evidence to support her belief in the paranormal, and feels a failure: will she ever get to be a famous parapsychologist? Meanwhile her acquaintances in the field win fame, fortune and university lectureships. Perhaps, they say, she doesn't believe fervently enough — her sceptical tendencies are interfering with her *ganzfeld*.

For all that, she has chosen the better part. Whilst others degenerated into writing pap astrology for station bookstalls (snake oil being temporarily out of fashion), she gradually came to understand that ESP, telekinesis, Tarot card readings and the whole shabby collection of spurious contacts with a deeper reality that make up parapsychology are born of a failure to grapple with the cruel demands of decent scientific method. They represent the last throw of Cartesian dualism against the notion that people are ultimately biodegradable. So instead she renovated a cottage, tried to pull a psychokinetic stunt with a home pregnancy test and got a job in television presenting a socially-aware, pop-science series.

So why write a book about it? The nearest we get to a real parapsychological phenomenon is the distinct impression that Dr Blackmore has more prospects as a ghost writer than a ghost hunter. More than half of the chapters in the book run to exactly ten pages — surely a significant result beyond that predicted by chance? And when one gets a real sniff of something worth knowing about — such as the repeated suggestion that Carl Sargent's Cambridge experiments were slightly less pure than the driven ectoplasm — substantiating detail is curiously unforthcoming. Why was she told never to darken the doors of his laboratory again?

There are gloomy lessons here for psychologists in general. Why, when psychological phenomena abound, poorly explained but going to the marrow of the welfare both of people individually and of society as a whole, should anyone try to base a career on the investigation of effects that no one can even find? Why has the statistical significance of experimental effects come to be seen as more important than their size? How can anyone survive an undergraduate course in Psychology, Philosophy and Physiology (as the author did) without acquiring a glimmer of philosophical insight into the business of scientific inquiry?

In favour of the author it has to be said that (the demands of the ghost writer or editor notwithstanding) she does not spare her own blushes over juvenilia, and one has the real sense that she stuck to method rather than yielding to peer group pressure. She found nothing worth reporting, and spends almost 250 pages telling us so.

Somewhere, waiting in the wings, are dewy-eyed undergraduates waiting to take up the torch of Rhine. Ouija boards at the ready, they will cling to their conviction that a mass of evidence for paranormal phenomena is unreasonably rejected by conventional scientists out of prejudice. Tarot card manufacturers have nothing to fear from this book. For whereas Dr Blackmore could have chosen to write a brave indictment, she has settled for self-indulgent apologia. ☐

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Highs and lows

George Fink

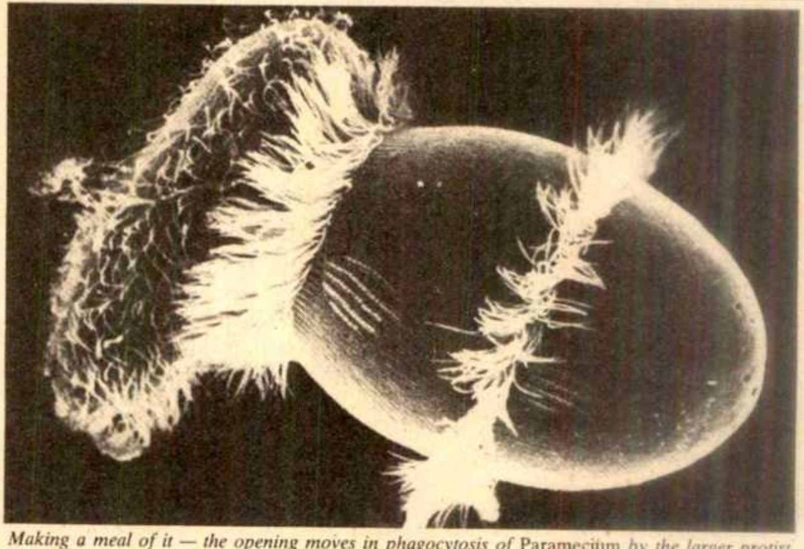
Drugs and the Brain. By Solomon H. Snyder. *W.H. Freeman: 1986. Pp.228. £15.95.**

SOLOMON Snyder's reason for entering medicine and medical research rather than becoming a student of philosophy was, as he states in his preface, that "philosophy is hardly a proper vocation for a nice Jewish boy". However, those who prefer the tight prose of Kurt Vonnegut may find Snyder's loquacious and at times awkward English a trifle heavy, and will be relieved that Snyder chose a career in medical research rather than in philosophy. Snyder probably entered a career in psychopharmacology because like many other boys, non-Jewish as well as Jewish (and not all "nice"), he found the study of the brain and the physical–chemical basis of the mind quite irresistible. This comes through clearly in *Drugs and the Brain*, which is by far the best book of its type that I have come across.

Using his formidable intellect, knowledge and resources, Snyder has produced a masterly work which explains clearly how drugs affect brain function. The general reader needs no more than an elementary knowledge of biology to understand the book, but at the same time *Drugs and the Brain* will delight the specialist. Snyder systematically covers all the psychotropic drugs — that is drugs which affect mood, thought, perception and behaviour — varying his approach from chapters which focus on a class of drugs (opiates, for example) to those which focus on particular psychiatric disorders, such as schizophrenia, and the drugs which are used in their treatment.

Each chapter starts with a lively historical account which concentrates upon key personalities involved in the discovery, synthesis, application or marketing of the drug concerned. For example, as well as dealing with the well-known account of how cocaine was the undoing of Sigmund Freud, Snyder tells the fascinating tale of the origin of the Coca-Cola Company. Angelo Mariani in 1863 patented 'Vin Mariani' a wine containing coca extract which soon became one of the most popular drinks in Europe. For this, and for developing coca lozenges and tea (used for dyspepsia and sore throat), Mariani received commendations and a special medal from Popes Pius IX and Leo XIII. John Pemberton, a pharmacist in Georgia, was inspired by Mariani's cocaine-containing preparations and in 1886 'designed' Coca-Cola as a stimulant and a treatment for headaches. Subsequently, Pemberton removed the alcohol from Coca-Cola, and the final formula for 'Classical Coke' was developed when, in 1888, Pemberton replaced ordinary water with soda water as the base. Asa Candler, another pharmacist bought the rights from Pemberton and founded the Coca-Cola Company in 1892. Cocaine was removed from the drink early in the twentieth century.

Snyder emphasizes that some drugs, although harmful in themselves, have formed the basis of drugs which have proved key armaments of modern medicine. Cocaine again serves as an excellent example. Its derivatives, Procaine, Lidocaine and Tetracaine, are potent local anaesthetics without which many modern surgical techniques could not easily be carried out. These anaesthetics have long been taken for granted and were originally synthesized from coca-leaves, but their success and popularity led drug companies to develop methods of synthesis which avoided the expense of importing coca-leaves. The 1980s scourge of cocaine might be partially alleviated and the livelihood of the Latin American peasants maintained if drug companies were persuaded to return to the older extraction methods which would require an almost complete diversion of all the coca-leaves from the synthesis of cocaine to the synthesis of local anaesthetics. Only a fool would believe that such a technical solution to a difficult social and economic problem is likely to be contemplated by politicians, let alone by the pharmaceutical companies and the major drug rings and their apparently respectable



Making a meal of it — the opening moves in phagocytosis of Paramecium *by the larger protist* Didinium nasutum. *The picture is reproduced from Peter H. Raven and George B. Johnson's introductory textbook* Biology, *which will be among the books reviewed in* Nature's *annual textbook review issue on 12 March.*

Gary Grimes/TAURUS Photos

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*In the United States published as a volume in the Scientific American Library.

dependents. However, Governments should at least be faced with the fact that there are several alternative equitable and moral solutions to the cocaine problem which do not necessarily require the destruction of peasant communities in the Andes.

The layout of the book is very attractive and some of the illustrations are strikingly beautiful. Classical coloured drawings of plants from which the various drugs are derived interleave with photographs of Inca ruins and reproductions of appropriate woodcuts, paintings and master drawings such as Albrecht Dürer's *Melencolia*. No expense appears to have been spared to reproduce in colour histological and histochemically prepared sections of the brain, clear diagrams of the relevant neural pathways in the brain and schematic illustrations of how drugs act. Included are diagrams of the structure of the various drugs, their derivatives and, where appropriate, their endogenous counterparts. There are also portrait photographs of many of the key figures such as John Cade, the Australian psychiatrist who discovered the value of lithium for the treatment of mania, and Nathan Kline who discovered that iproniazide, a monoamine oxidase inhibitor that was first used in the treatment of tuberculosis, is a potent antidepressant. Real data are used judiciously to back up the simplified explanations in the text and figures.

If *Drugs and the Brain* has any fault it is that it entrenches the view that each neurotic and psychotic symptom is due to the malfunction of one specific chemical neurotransmitter system. However, this is the age of chemical phrenology, and so in a book written for the non-specialist it is indeed very difficult to paint a picture which is clear but which at the same time is sufficiently sceptical of the notion that each mood, feeling, perception or behaviour is mediated by one specific neurotransmitter.

Solomon Snyder has produced a book which will be enjoyed by anyone with an interest in one of the most dramatic and fascinating advances in biology, the way neurons communicate to produce the properties of what we call the mind and how these communications are altered by drugs. *Drugs and the Brain* should be read and understood by politicans, civil servants and members of committees responsible for dealing with the enormous social and economic problems caused by drugs that affect the brain. The specialist too will wish to purchase the book. Not only will it look very fetching on the coffee-table now, but it could well become a collector's item in 25 years time. ☐

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How well fed is the world?

Dennis T. Avery

People, Food, and Resources. By Kenneth Blaxter. *Cambridge University Press: 1986. Pp. 118. £15, $29.95.*

Technology in the 1990s: Agriculture and Food. Edited by Kenneth Blaxter and Leslie Fowden. *The Royal Society: 1986. Pp. 179. Distributed by Cambridge University Press, £32.50, $54.50.*

In *People, Food, and Resources* Sir Kenneth Blaxter makes a serious, well-intentioned effort to assess the dangers of rising world population and limited food supply. He concludes that the outlook is poor because continued high population growth is certain and increased food production is not. For all the good intentions, however, both Sir Kenneth and the contributors to the Royal Society volume which he co-edited leave their readers struggling in the Club of Rome's ignorance *circa* 1974 — the report of that date predicted that famine was soon to sweep the world. The books do not reveal that in the 1980s the world's food production is expanding even faster than its fast-growing population and may still be picking up speed.

According to the statistics of the UN Food and Agriculture Organization (never known for minimizing the problem of hunger in the world) per capita food production in the developing countries gained 17 per cent in the period 1980 to 1984 alone. The developing countries raised their farm output by 38 per cent during the decade 1974–1984, and 4.1 per cent annually in the 1980s. This is a much more rapid increase than the historic 2.5 per cent per year cited by Sir Kenneth. Virtually all of the recent increase in farm output in less-developed countries came from higher yields rather than extending plantings onto fragile acres or cutting down forests. This is an astounding human achievement, one which readers will not winnow out from Sir Kenneth's determined pessimism and bias towards population management.

Sir Kenneth can be forgiven part of his dour outlook; the population growth curve has long been the least tractable element of the food–population equation. But most population experts now concede that the curve is S-shaped rather than L-shaped. As the developing nations become more affluent, their population growth will slow. Sir Kenneth says in *People, Food, and Resources* that he sees no reason to expect this to occur — but most of us know that in modern societies additional children cease to add wealth or social insurance. They become instead a major net expense to the family.

In his most interesting chapter, Sir Kenneth offers a nice tribute to the much-maligned Reverend Malthus. Then he proceeds to follow Malthus into the trap of assuming that population will always outrun food supply — and there is now far more reason to believe in the potential fruits of agricultural research than there was in 1800.

To Sir Kenneth's credit, he apparently made a legitimate effort to find out the prospects for increasing world food production. But his specialists failed him. *Technology in the 1990s: Agriculture and Food*, the report of The Royal Society's conference on food production, lacks perspective, is highly uneven in quality and the issues concerned are obscured by over-technical language. Take this, for example: "Peptides corresponding to the antigenic site of food-and-mouth disease virus which have been synthesized either by the solid-phase method or as a part of the fusion protein have elicited neutralizing antibody and protect cattle against challenge infection . . .". The passage hardly informs the lay reader that the first, fully safe vaccine for foot-and-mouth disease has now been produced through genetic engineering.

We should not be surprised, however. Researchers have not made a good job of public liaison in the dozen years since the Club of Rome report. They should have been telling us about the powerful new techniques in plant genetics and the farming systems that are transforming agriculture in the less-developed countries, and about the potential of new lines of research. Perhaps they feared overstatement, or believed that a rush of public concern would be good for their budgets. Their lack of communication — and our resulting ignorance — has been expensive for everybody. Farmers in the affluent countries have been misled about the value of their land and the need for their produce. Consumers and taxpayers throughout the OECD countries have paid dearly to finance farm surpluses the Third World doesn't need. And few of the less-developed countries themselves have understood their farming opportunities clearly. These two books won't do much to help any of them. ☐

Dennis T. Avery is Senior Agricultural Analyst in the US Department of State, Washington, DC 20045, USA.

- Just published by W.W. Norton is the fourth of the *State of the World* reports from the Worldwatch Institute. Like its predecessors, the 1987 edition "examines the counterpoint of urgency and uncertainty that has come to dominate world affairs in an age when the environmental consequences of human activities transcend national boundaries"; included are chapters on, among other topics, world agriculture, nuclear power, re-cycling of waste materials and the chemical cycles. Price is hbk $18.95, pbk $9.95.

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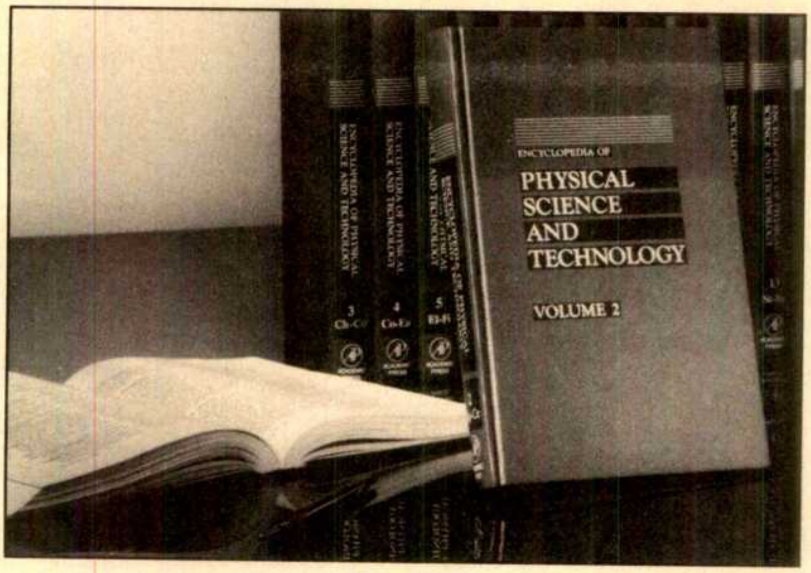

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The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing

Tom Maniatis & Robin Reed

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A small set of distinctive short RNA molecules are found in the nuclei of all higher eukaryotic cells and yeast, in protein complexes known as 'small nuclear ribonucleoprotein particles', or snRNPs. Recent work has confirmed early suggestions that these particles form part of the machinery by which primary RNA transcripts are processed to their mature, functional form. In particular, snRNPs have been shown to be an integral part of the 'spliceosome', a multi-component complex involved in the removal of intron sequences from the coding regions of messenger RNA precursors.

THE precise excision of intervening sequences during RNA splicing is an interesting example of the high degree of specificity involved in biosynthetic processes. Self-splicing RNA precursors achieve this specificity primarily through intramolecular interactions[1] whereas all other types of RNA splicing require interactions between cellular factors and specific recognition signals in the RNA precursors. The primary candidates for the cellular recognition factors involved in splicing of mRNA precursors (pre-mRNA) are the small nuclear ribonucleoprotein particles (snRNPs). This notion was initially based on the observation that sequences at the 5′ end of the RNA of one Sn RNP, U1 RNA, are complementary to the consensus 5′ splice site[2-4]. Although experimental evidence implicating snRNPs in pre-mRNA splicing has accumulated during the past five years, definitive proof was obtained only recently.

Much of the recent progress in elucidating the function of mammalian snRNPS has resulted from the development of efficient *in vitro* splicing systems[5-7]. Both normal and mutant RNA precursors can be synthesized in large amounts using plasmids containing the bacteriophage SP6 promoter[8,9], and these precursors are accurately spliced in nuclear extracts[7]. Interactions between snRNPs and pre-mRNA have been studied during the splicing reaction using a variety of approaches including the use of antibodies directed towards either the protein components of snRNPs[10] or the trimethylguanosine cap of the snRNAs[11,12]. In addition, targeted nuclease digestion of specific snRNAs and the purification of splicing complexes have provided new insights into the function of snRNPs.

Substantial progress has also been made recently in the analysis of yeast pre-mRNA splicing and in the characterization of yeast snRNPs. The purpose of this review is to summarize the evidence showing that snRNPs play an essential role in mammalian and yeast pre-mRNA splicing, and to discuss recent advances in understanding the interactions between snRNPs and pre-mRNA.

Mammalian splicing and snRNPs

The snRNPs are ribonucleoprotein particles found in the nuclei of all higher eukaroytic cells (see refs 13 and 14 for reviews). snRNPs consist of one snRNA molecule, ranging in size from 6 to 217 nucleotides, and a set of approximately 10 different proteins. Some of these proteins are unique to a particular snRNP whereas others are common to all particles. The major species, U1, U2, U4, U5 and U6 are nucleoplasmic, are present at an abundance of 200,000 to 1,000,000 copies per cell, and contain a trimethylguanosine cap (with the exception of U6 snRNA which contains an unidentified cap structure). In addition, these snRNPs are associated with the Sm antigen, which is recognized by sera from patients with autoimmune disease[10]. Most snRNPs are isolated as monomeric particles, but U4 and U6 are recovered from cells as a single entity[15,16]. A fraction of the U1 and U2 snRNPs also appear to be complexed with each other[17]. Recently, four new snRNPs were discovered. Designated U7[18], and U8-U10[19], they were not previously detected because of their relatively low abundance. It is possible that additional snRNAs are present in mammalian cells but have eluded detection.

The first experimental evidence that snRNPs are involved in splicing was provided by antibody inhibition studies. Anti-snRNP antibodies were shown to inhibit splicing in isolated nuclei[20], and subsequently in splicing extracts[21,22]. Unfortunately, these studies did not provide unequivocal evidence for a role of snRNPs in splicing because the addition of partially purified snRNPs to antibody-depleted extracts did not restore splicing activity[22].

A more direct demonstration that snRNPs play a role in splicing was provided by experiments in which the RNA component of snRNPs was inactivated by nuclease digestion. Treatment of splicing extracts with micrococcal nuclease, followed by EGTA inactivation of the nuclease, completely inhibits RNA splicing. In contrast to the antibody-inhibition studies, splicing activity could be restored to nuclease-treated extracts by the addition of partially purified snRNPs which are themselves devoid of splicing activity[23]. To demonstrate involvement of individual snRNP types in the splicing reaction, targeted nuclease digestion was used to inactivate specific snRNAs. This was accomplished by hybridizing synthetic DNA oligonucleotides to specific sequences in snRNAs and then treating the DNA-RNA hybrid with the enzyme ribonuclease (RNase) H which degrades the RNA strand of the RNA-DNA duplexes[22]. This approach was used initially to show that RNase H cleavage of the 5′ end of U1 snRNA inhibits splicing[22-24]. Similar RNase H cleavage experiments were subsequently employed to demonstrate a requirement for U2 (refs 23, 24), U4 (refs 25, 26) and U6 (refs 26) snRNPs in the splicing reaction. Splicing activity could be reconstituted by combining extracts in which different snRNAs were inactivated[23-26]. Thus, the inhibitory effect of RNase H cleavage could be directly linked to the inactivation of specific snRNAs.

Progress in understanding the mechanism of pre-mRNA splicing led to more-specific questions regarding the role of snRNPs in the splicing reaction. Studies *in vitro* revealed that splicing proceeds by a two-step mechanism (refs 7, 27-29; see ref. 30 for review). The first step entails cleavage at the 5′ splice site and the formation of a 2′-5′ phosphodiester bond between the 5-terminal G residue of the intron and an A residue located ~30 nucleotides upstream from the 3′ splice site. The second step involves cleavage at the 3′ splice site, ligation of the two exons, and release of the excised intron as a lariat. Sequence comparisons[28,31,32] and functional studies[33-35] indicated that, in addition to the conserved 5′ and 3′ splice junction sequences, the site of lariat formation is a recognition element for pre-mRNA splicing. Indeed, the branchpoint sequence is protected from oligonucleotide-directed RNase H cleavage and RNase A digestion during the splicing reaction[36]. This protection is not observed in the absence of ATP or with precursors containing splicing mutations. A possible function for snRNPs in branchpoint protection was suggested by the observation that binding to the branchpoint sequence is not detected in extracts treated with micrococcal nuclease[36].

Specific interactions

The first demonstration that snRNPs can interact specifically with pre-mRNA was provided by experiments showing that extensively purified U1 snRNP binds to the 5′ splice site of an *in vitro* synthesized mouse β-globin pre-mRNA[37]. Significantly, snRNP proteins are required for binding specificity. In more recent experiments, purified U1 snRNP was shown to bind specifically to 5′ splice-site sequences in RNA[38]. Surprisingly, specific binding was also observed with single-stranded DNA containing 5′ splice-site sequences. A low level of 3′ splice-site activity was observed with U1 snRNP, but the functional significance of this interaction, if any, is not known.

When *in vitro* splicing systems were developed, interactions between specific snRNPs and pre-mRNA could be analysed during the splicing reaction. This was accomplished using an RNase protection and immunoprecipitation assay in which labelled pre-mRNA is added to an *in vitro* splicing extract, incubated under splicing conditions, and digested with RNase T1 in the presence of anti-snRNP antibodies[12,24]. Regions of the pre-mRNA that interact specifically with snRNPs are protected from nuclease digestion and precipitated by the anti-snRNP antibodies. This approach was used to show that U1 snRNP binds to the 5′ splice site and that U2 snRNP binds to the branchpoint sequence[24]. These splicing recognition sequences are not protected from RNase T1 cleavage if the snRNPs are inactivated by oligonucleotide-directed RNase H cleavage. An additional snRNP, possibly U5, interacts specifically with the 3′ splice site[12]. The functional significance of the specific interactions between snRNPs and pre-mRNA was demonstrated by showing that RNase protection is not observed with pre-mRNAs containing mutations in the splicing recognition elements[12,24].

The biochemical requirements for the binding of different snRNP types to their recognition sequences vary. Specific binding to the 5′ and 3′ splice sites is observed immediately after the pre-mRNA is added to the extract at 0 °C, and ATP is not required[12,24]. In contrast, binding to the branchpoint sequence requires incubation at 30 °C in the presence of ATP[12,24,36]. These observations suggest that the first event in the splicing reaction is binding to the 5′ and 3′ splice sites. Binding to the branchpoint sequence then occurs as the assembly of the splicing complex proceeds.

Genetic evidence for base pairing

As discussed above, three different snRNPs have been implicated in site-specific binding to pre-mRNA (U1, U2, and U5). The observed binding specificities may involve base-pairing interactions between the snRNA and pre-mRNA, such as that proposed for U1 snRNA[2-4], and/or snRNP proteins may mediate specific binding to pre-mRNA. Although models for base-pairing between U2 snRNA and pre-mRNA have been proposed[39,40], the suggested complementarity is less striking than that observed with U1 snRNA. Finally, examination of the U5 snRNA sequence failed to reveal significant complementarity with the 3′ splice-site consensus sequence[12].

The first evidence for base-pairing interactions between U1[41] and U2[42] snRNPs and high-molecular-mass nuclear RNA was provided by *in vivo* psoralen cross-linking studies. Because specific binding to splicing recognition sequences was not demonstrated, these studies did not provide an experimental link between base-pairing and splicing. More recently, this link was demonstrated by a direct genetic test[43]. The experiments were designed to determine whether point mutations in the 12S and 13S 5′ splice sites of the adenovirus *Ela* gene, which disrupt potential base-pair interactions, can be suppressed by compensatory base changes in human U1 snRNA. Plasmids containing the mutant *Ela* genes and either the wild-type or suppressor mutants of the U1 gene were transfected into HeLa cells. Analysis of RNA obtained from the transfected cells revealed that a mutation at position +5 in the 12S splice site was efficiently suppressed by U1 RNA containing the compensatory base change. The potential base-pair interactions between the wild-type and mutant 12S 5′ splice sites and U1 snRNA are illustrated in Fig. 1. Interestingly, increasing the complementarity between the wild-type 12S 5′ splice site and U1 snRNA by introducing a single base change into U1 RNA led to more efficient use of the 12S splice site in the wild-type *Ela* RNA precursor. Thus, one of the important determinants in 5′ splice site recognition appears to be the extent of complementarity between U1 snRNA and the 5′ splice site. Although these studies indicate the base-paring between the 5′ splice site and U1 snRNA is necessary for splicing, this interaction alone is not sufficient since suppression could not be achieved with a splicing mutation at +3 in the 12S splice site[43]. The possibility that protein–RNA interactions are involved in specific snRNP binding to pre-mRNA is suggested by the identification of an snRNP protein that binds specifically to the 3′ splice-site sequence[44-46].

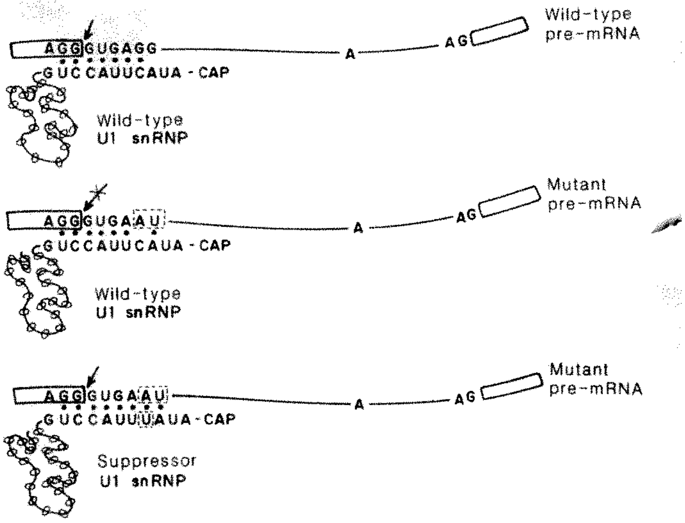

Fig. 1 The suppression of a mutation in the adenovirus *Ela* 5′ splice site by a compensatory base change in U1 snRNA. The pre-mRNA is represented by the open boxes (exons) and thin lines (introns). The *Ela* 12S 5′ splice site, branchpoint sequence and 3′ splice site are indicated by GUGAGG, A and AG, respectively. Wild-type U1 snRNA is capable of forming 7 contiguous base pairs with the 5′ splice site. In the mutant pre-mRNA, one of these base pairs is disrupted by the G-to-A change at position 5. Changing the C at position 4 of U1 snRNA to a U results in the re-establishment of base pairing at position 5 of the mutant pre-mRNA, and suppression of the splicing mutation[43]. The arrow indicates the site of cleavage at the 5′ exon intron junction and the base changes in mutant pre-mRNA and the suppressor U1 snRNA are indicated by the open boxes with the dashed lines.

Splice-site selection

Correct splice-site selection requires discrimination between bona fide splice sites and the many sequences in RNA precursors that closely resemble or are identical to consensus 5′ and 3′ splice sites (pseudo-splice sites). These pseudo-sites are never used in the presence of the normal splice sites, but at least some of them (cryptic splice sites) are used efficiently when the normal sites have been inactivated by mutation[47,48]. These observations indicate that the consensus splice-site sequences are necessary but not sufficient for accurate splicing. The second aspect of

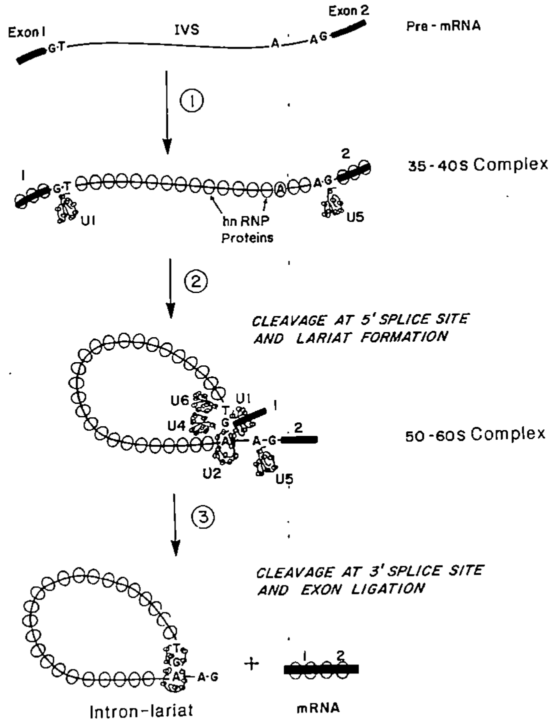

Fig. 2 A model for spliceosome assembly. In the first step a 35-40S complex is formed in which U1 and U5 snRNPs bind to the 5' and 3' splice sites respectively, and hnRNP proteins bind at unspecified locations. In the second step, U5 snRNP binds to the 3' splice site and cleavage at the 5' splice site and lariat formation occurs. The site at which the U4 and U6 snRNP complex binds is not known. The complex thus form sediments at 50-60S. In the final step, cleavage at the 3' splice and exon ligation leads to the release of the intron as a lariat containing factors bound to the branchpoint sequence.

splice-site selection involves the mechanism by which the appropriate pairs of 5' and 3' splice sites are chosen in mRNA precursors containing multiple introns. Although the splice sites in different introns of the same precursor are nearly identical, pairing of a 5' splice site in one intron with a 3' splice site in another is rarely observed in normal pre-mRNAs. Thus, the splice-site sequences alone are not sufficient to specify correct splice-site pairing in multi-intron RNA precursors.

Based on the demonstration of specific interactions between snRNPs and splice-site recognition sequences described above, snRNPs are likely to be involved in both aspects of splice-site selection. Considering that the splicing recognition sequences alone are not sufficient to specify correct splice-site selection, other sequences must be involved. Progress in identifying these sequences was recently provided by the analysis of precursors containing duplicated 5' or 3' splice sites[49]. These experiments revealed that deletions, substitutions or even subtle changes of exon sequences can affect the use of adjacent splice sites. In many cases these changes result in the complete inactivation of the splice site adjacent to the altered exon. The nature of the sequences within the normal flanking exons required for the efficient use of the adjacent splice site is not known. Higher-order structure of the exons may facilitate binding of snRNPs or other splicing factors to splice sites. Alternatively, the normal exon sequences may be required for optimal splicing complex formation and stability.

Experiments with the splice-site duplication precursors may also be relevant to the mechanism of correct pairing of 5' and 3' splice sites in precursors containing multiple introns. In particular, the data indicate that when duplicated splice sites are flanked by identical sequences, the pair of 5' and 3' splice sites in closest proximity to one another are preferentially used[49].

Spliceosomes and snRNPs

Understanding the detailed mechanisms of pre-mRNA splicing will require the identification and purification of individual splicing components. However, progress in this direction has been hampered by the complex nature of the splicing apparatus. Several factors other than snRNPs, including heterogeneous nuclear RNP (hnRNP) proteins[50], appear to be required for the splicing reaction[23,51-53]. Preliminary fractionation of splicing components by column chromatography was made possible by the development of *in vitro* complementation assays[23,51-53]. However, difficulties in recovering sufficient quantities of active splicing factors have impeded progress using this approach.

An alternative approach for isolating splicing components is to identify factors that specifically interact with the 5' splice site, 3' splice site, or the branchpoint sequence[44-46]. Two factors identified in this way may mediate interactions between snRNPs and their recognition sequences in pre-mRNA. One factor enhances the binding of U1 snRNP to the 5' splice site[44]. Another factor, which binds specifically to the 3' splice site, can be immunoprecipitated by anti-Sm monoclonal antibodies[45,46]. Interestingly, the binding activity co-fractionates with U5 snRNP[45], which is consistent with the proposal that this snRNP binds to the 3' splice site[12]. This factor can be dissociated from snRNPs with high concentrations of Mg^{2+}, but the dissociated factor retains splice-site binding activity[45]. Thus, the specific binding of U5 snRNP to pre-mRNA may be mediated by protein-RNA interactions, rather than by base-pairing.

Splicing components have also been identified using techniques designed to purify the entire splicing complex, designated the spliceosome[54-56]. Spliceosomes were originally detected by adding labelled RNA precursors to splicing extracts, incubating under conditions that are optimal for splicing, and then sedimenting the reaction mix through a sucrose or glycerol density gradient[54-58]. Although considerable variability has been reported in the sedimentation coefficients of the complexes and the kinetics of their formation, it is generally agreed that two classes of complexes can be observed (Fig. 2). One complex, which sediments at 35-40S, forms at 0 °C in the absence of ATP and its formation does not require functional 5' and 3' splice sites in the pre-mRNA. A 35-40S complex is also the first particle observed at 30 °C in the presence of ATP. As the splicing reaction proceeds, a second complex is observed at 50-60S. Its formation requires ATP, incubation at 30 °C and normal 5' and 3' splice sites. Depletion of hnRNP proteins from the splicing extract[47] or inactivation of snRNPs[55,56,58] prevents the formation of this complex. Finally, both unspliced pre-mRNA and splicing intermediates are found in the gradient fractions containing this complex. The 50-60S complex is missing one or more essential components, since incubation of the sucrose gradient fractions in splicing buffer does not produce additional splicing products. However, when splicing extract is mixed with the complex, splicing products are generated and the reaction is accelerated[56].

A hypothetical pathway for spliceosome assembly is shown in Fig. 2. This pathway, which is based on the sequence and factor requirements for spliceosome assembly *in vitro* and on RNase protection experiments, provides a useful working model, but may or may not reflect the actual steps involved in spliceosome assembly *in vivo*. For example, because a precursor-product relationship has not been established, the 35-40S complex could be an *in vitro* dead-end complex rather than a bona fide precursor.

Purification of intact spliceosomes has been difficult because they appear to aggregate and associate with non-specific proteins and RNA in the nuclear extract. In an attempt to circumvent these problems, the polyanion heparin was added to the splicing reaction after spliceosome formation[59,60]. However, this treatment decreases the sedimentation coefficient of the spliceosome from 60S to 35S, suggesting that specific as well as non-specific components may have been dissociated. These 'core' complexes have been characterized by both gel electrophoresis[59] and affinity chromatography[60]. In the latter approach, a small number of biotinylated nucleotides were incorporated into an RNA precursor. This precursor was then incubated in a splicing extract, treated with heparin, fractionated on a glycerol gradient, and finally, purified by selection on streptavidin agarose beads. Analysis of the small RNAs associated with the purified 35S core complexes revealed the presence of U2, U5, U4 plus U6 snRNAs, and an unidentified RNA species, X. The conspicuous absence of U1 snRNA from the complex could be a result of the heparin treatment, or U1 snRNP may be bound to the splicing complex only transiently[60]. Significantly, quantitative analysis of the snRNAs associated with the complex indicated that each snRNP is present at roughly one copy per pre-mRNA.

Although the characterization of spliceosomes has provided interesting insights into the nature of the components associated with the splicing complex, the preparative purification of intact, functional spliceosomes has yet to be achieved. Conditions must be found which prevent aggregation and non-specific binding to nuclear components, but do not inactivate the spliceosome. On the basis of the information obtained thus far, the spliceosome appears to be a dynamic structure assembled on the pre-mRNA template, possibly in a stepwise fashion, and then dissociated as the splicing reaction is completed. Purification of a homogeneous population of splicing complexes may require blocking the splicing reaction at different steps. An important point to emphasize is that the components involved in spliceosome assembly are freely exchangeable since extracts in which different splicing factors have been inactivated efficiently complement each other *in vitro*. Like the ribosome, the spliceosome appears to be composed of relatively stable subunits (the snRNP particles), and many factors that are involved in the assembly of an active complex on the RNA substrate. Purification of functional splicing complexes may therefore prove to be a significant challenge.

Yeast pre-mRNA splicing

Advances in understanding yeast pre-mRNA splicing have paralleled those achieved in mammalian systems. Both *in vivo*[61-63] and *in vitro*[63,64] studies with the yeast *Saccharomyces cerevisiae* have shown that the two-step splicing mechanism established in mammalian systems is also used in the processing of yeast nuclear pre-mRNAs. In addition, a number of studies have demonstrated the importance of the 5′ and 3′ splice sites and the branchpoint sequence in yeast pre-mRNA splicing[65-72]. Finally, preliminary biochemical fractionation studies have begun to identify splicing factors[73], and density gradient sedimentation has shown that splicing occurs in a 40S spliceosome[54].

In spite of many similarities between yeast and metazoan splicing, a number of differences have been noted. First, the yeast 5′ splice site and branchpoint sequences are highly conserved although the corresponding sequences in metazoan pre-mRNAs vary significantly[65-67]. Second, mutations in the yeast splice sites and the branchpoint sequence usually inactivate splicing[65-67] whereas the corresponding mutations in mammalian introns usually lead to the use of cryptic sites[33,35,48,49]. Third, unlike metazoan pre-mRNAs, most yeast 3′ splice sites do not contain an extensive polypyrimidine tract[69,72], and other evidence suggests that the polypyrimidine sequences may not be essential for cleavage at the 5′ splice site and lariat formation *in vitro* [72]. In contrast, the first step in splicing does not occur when the 3′ splice site is deleted in mammalian introns[33,55,74]. Although mammalian mRNA precursors are not spliced in *Saccharomyces cerevisiae*[75,76], yeast pre-mRNA is accurately spliced in mammalian cell extracts. However, the lariat forms at a different site[77]. Taken together, these observations suggest that the sequence elements at the branchpoint and the 3′splice site may have somewhat different functions in yeast and metazoan splicing.

The differences in sequence requirements for splicing in yeast and metazoa may be a consequence of the way genes are organized and expressed in the two types of organisms. Although a few genes in *S. cerevisiae* contain one or two small introns, most are intron-free. In contrast, the majority of higher eukaryotic genes contain multiple introns, which range in size from 30 to several thousand nucleotides. A number of higher eukaryotic genes also encode transcripts that are differentially spliced[30]. The mechanisms required to accurately remove multiple introns from a single RNA precursor without exon deletion, and to regulate cell-specific differential splicing, may have necessitated greater flexibility in the sequence requirements for RNA splicing in higher eukaryotes.

Until recently, functional homologue of mammalian snRNPS had not been identified in yeast. Yeast snRNPS could not be immunoprecipitated with anti-Sm antibodies[2] nor detected by cross-hybridization with clones snRNA genes[78-82]. However, at least two different yeast snRNAs contain binding sites for the Sm antigen, since these snRNAs can be immunoprecipitated with human anti-Sm antibodies after the snRNAs are injected into *Xenopus* oocytes[83]. Thus, the specific interaction between the Sm-antigen and yeast snRNAs has been conserved, but the yeast Sm-antigen is not recognized by antibodies raised against the mammalian analogue. Although yeast cells contain at least 24 different snRNAs with a tri-methyl cap at their 5′ termini[82], these RNAs differ significantly from their presumed metazoan counterparts. Yeast snRNAs are encoded by single-copy genes[79,80], are present at only 10–500 copies per cell[78] and range in size from 120 up to 1,000 nucleotides[82]. Attempts to investigate the function of yeast snRNAs, and possibly establish a link to pre-mRNA splicing, proved difficult because gene disruption techniques revealed that many of the snRNA genes are not essential for growth[79-81]. This observation suggested that the gene products are either functionally redundant or that they are not essential for splicing.

The development of a yeast *in vitro* splicing system[61,63-64] provided the opportunity to investigate the involvement of snRNPs in pre-mRNA splicing. A function for snRNPs was first suggested by the observation that yeast splicing extracts are inactivated by treatment with either micrococcal nuclease or trimethyl-cap antibodies[73]. In subsequent experiments, the association of specific snRNAs with the splicing complex was demonstrated by analysis of spliceosomes purified by gel electrophoresis or affinity chromatography[84]. When yeast spliceosomes were treated with high concentrations of salt, EDTA and large amounts of nonspecific carrier RNA to disrupt nonspecific RNA-protein interactions, three distinct complexes were separated by gel electrophoresis[85]. Analysis of the snRNA molecules associated with the three complexes revealed that the first complex formed is associated with an snRNA greater than 1000 nucleotides in length, designated large spliceosomal RNA1 (LSR1)[85] or snR20[82]. The formation of this complex precedes cleavage at the 5′ splice site and lariat formation. The next complex formed contains three different small snRNAs designated snR7S, snR7L and snR14[82], in addition to LSR1. The final complex contains LSR1, snR7S, snR7L, but is missing snR14. Both the splicing intermediates and products are found in the final complex, suggesting that the release of snR14 precedes cleavage at the 3′ junction and exon ligation. Although this putative pathway of yeast spliceosome assembly provides a useful working model, it is important to emphasize that the spliceosome was treated with high salt, EDTA and nonspecific competitor RNA before gel electrophoresis. Therefore, the electrophoretic behaviour of the splicing complexes and the differences in the associated snRNAs may reflect the conditions used to analyse the complexes. Moreover, there is no direct evidence for a precursor–product relationship between the different complexes.

Recently, a surprising relationship between the 1,000-nucleotide yeast snRNA LSR1 and mammalian snRNPs was serendipitously discovered[86]. In a negative control for U2 transcrip-

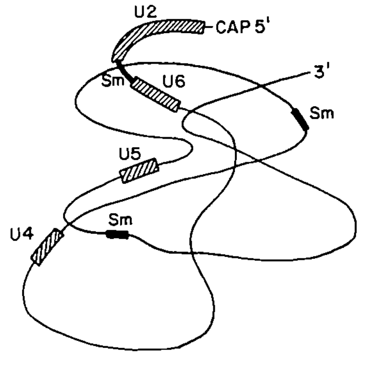

Fig. 3 The organization of the yeast LSR1 snRNA. Hatched boxes, regions homologous to sequences that are highly conserved in different mammalian snRNAs; thick black lines, possible binding sites for the Sm antigen[86].

tion experiments, an oligonucleotide complementary to U2 snRNA was used to prime cDNA synthesis with total yeast RNA. Unexpectedly, a cDNA was synthesized, and its sequence was found to be nearly identical to human U2 snRNA. An RNA larger than 1,000 nucleotides in length was detected when the human U2 oligonucleotide was used to probe a Northern blot of yeast RNA. Human U2 snRNA is only 189 nucleotides in length. When the gene encoding the large yeast RNA was cloned and sequenced, not only was extensive homology to human U2 snRNA detected, but short stretches of sequences were observed that are highly conserved in metazoan U4, U5 and U6 snRNAs (Fig. 3). Although these observations raise the interesting possibility that LSR1 is a 'poly snRNP' equivalent to a complex of U2, U5 and U4/U6 snRNPs in metazoans[87], the regions of homology are very short, and they are not found in secondary structures that are highly conserved in metazoan snRNAs. Elucidating the function of the unusually large LSR1 snRNA must await the analysis of point mutations in the conserved regions, and the characterization of other snRNAs associated with the yeast spliceosome.

The association of LSR1 with the spliceosome and its sequence homology to mammalian snRNAs, strongly suggest that it is involved in splicing. Further evidence was provided by gene disruption experiments showing that the LSR1 gene is essential for yeast viability[86]. An interesting aside is that LSR1 is present in *Saccharomyces cerevisiae*, but not in *Schizosaccharomyces pombe*[86], where the human U2 hybridization probe detects an RNA that is approximately the same size as human U2 RNA. This observation may correlate with the finding that metazoan pre-mRNAs are not spliced in *Saccharomyces cerevisiae*, but at least one mammalian pre-mRNA is spliced in *Schizosaccharomyces pombe*[87]. However, since different mammalian pre-mRNAs were tested in the two species, a generalization at this point may be premature. In any case, a number of differences between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been noted, including the role of *ras* proteins, cell cycle control and transcriptional regulation. In all of these examples, *Schizosaccharomyces pombe* appears to be more closely related to metazoa than *Saccharomyces cerevisiae*[88].

All the elements necessary for a concerted biochemical and genetic analysis of yeast pre-mRNA splicing are now in place. In addition to identifying and characterizing the genes encoding the snRNAs involved in splicing, a number of other genes required for splicing have been identified. A class of temperature-sensitive mutants designated *rna*[89,90] were shown to be defective in nuclear pre-mRNA splicing[91,92]. Definitive evidence that at least three of the *rna* gene products are directly involved in pre-mRNA splicing was recently provided by *in vitro* complementation experiments[93]. In these studies, splicing extracts prepared from mutant cell lines were shown to be temperature sensitive (ts) for splicing. These extracts can be complemented by heat-treated extracts prepared from different ts mutants[93]. Thus, it is only a matter of time before many of the genes essential for yeast pre-mRNA splicing are cloned and characterized, and their products produced in amounts suitable for detailed biochemical studies.

The role of snRNPs in 3′ end processing

The snRNPs have also been implicated in other types of RNA processing. For example, specific interactions between snRNPs and pre-mRNAs play a role in 3′ end formation of histone mRNAs (see ref. 94 for review). In this case, U7 snRNA base-pairs with a 3′ processing signal located within the histone mRNA precursor[95]. The functional significance of the base-pair interactions was recently demonstrated by showing that mutations in the 3′ recognition element could be suppressed by compensatory base changes in U7 snRNA[95].

There may also be a function of snRNPs in 3′ end processing of polyadenylated mRNAs. This possibility is supported by the observations that accurate *in vitro* polyadenylation is inhibited by anti-Sm and anti-U1 antibodies[96], and that the AAUAAA sequence required for polyadenylation associates with a factor that is precipitated by anti-Sm or anti-trimethyl cap antibodies[97]. However, oligonucleotide-targeted RNase H digestion of U1, U2, U4 snRNAs alone or in combination has no effect on *in vitro* cleavage or polyadenylation[25,97]. Even more puzzling is the observation that micrococcal nuclease treatment of nuclear extracts prevents 3′ end formation[97,98], but the activity can be restored by the addition of purified RNA from a variety of sources[98]. Thus, the *in vitro* polyadenylation reaction appears to have a general requirement for RNA mass, and possibly an snRNP other than U1, U2 or U4. At present, the antibody-inhibition and nuclease-digestion data cannot be reconciled.

Possible mechanisms of snRNP action

The mechanism by which specific interactions between snRNPs and pre-mRNAs lead to site-specific cleavage or ligation is not known, but a number of interesting possibilities exist. First, snRNPs may be 'ribozymes', by analogy with RNase P where the RNA component of a ribonucleoprotein complex is able to cleave transfer RNA precursors at a specific site[99]. Another analogy is the ribosome: recent studies have shown that a binary complex of 16S and 23S ribosomal RNA is capable of catalysing many of the steps of protein synthesis in the presence of a limited number of ribosomal proteins[100]. This observation raises the interesting possibility that the catalytic function of ribosomes is provided by ribosomal RNA whereas ribosomal proteins merely serve to finely tune the structure and activity of the RNA[1,101]. Second, snRNPs may be a part of a scaffold structure that holds the appropriate regions of the pre-mRNA in close proximity so that the splicing event occurs without the involvement of protein enzymatic activity. This is suggested by the observation that class II yeast mitochondrial mRNA precursors are self-splicing, and the mechanism involves a lariat intermediate[102,103]. Thus, both types of pre-mRNA appear to be spliced by the same mechanism, but one requires snRNPs and the other does not. The function of snRNPs in nuclear pre-mRNA splicing may therefore be served by intramolecular interactions in self-splicing class II mitochondrial pre-mRNAs. Third, snRNPs may be trafficking particles that carry the appropriate enzymes to their specific sites of action. For example, endonucleases and ligases may be bound to snRNPs. Although these alternatives cannot be distinguished now, the stage is set for determining the precise role of snRNP particles in mammalian and yeast pre-mRNA splicing.

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ARTICLES

Topography of the core–mantle boundary and lateral homogeneity of the liquid core

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Separate inversions of travel-time residuals of waves reflected from and transmitted through the core–mantle boundary yield similar results in terms of spherical harmonic expansion of its topography up to degree and order 4. This indicates absence of detectable lateral heterogeneity in the liquid core. The relief of the boundary is ±6 km but its flattening, determined by the c_2^0 term, does not depart significantly from the hydrostatic theory.

IT is now possible to address the question of aspherical structure of the Earth over the range of depths from its centre to the surface. This is being accomplished using data that include waveforms of body and mantle waves, travel times of seismic pulses and decomposition of the fine structure of spectral peaks of free vibrations of the Earth. Information contained in these data sets is not independent, and the results of the interpretation can be verified by comparing the derived models. For example, Giardini *et al.*[1] in their Figs 3 and 4 compare models of the lower mantle obtained using three very different sources of data: splitting of normal modes[1], waveform inversion of body wave and mantle wave data[2] and travel time residuals[3] (see the cover of this issue). The agreement between models derived from different kinds of data stops above the core–mantle boundary (CMB), except that the available information indicates the presence of lateral heterogeneity below that level[1,3–9].

The CMB is the most dramatic discontinuity in the Earth's internal structure, concerning physical and chemical properties as well as the timescale of the processes that take place on either side of it. Retrieval of its shape is important not only for our being able to address, for example, the question of lateral heterogeneity in the inner core, but also for our understanding of the geodynamic processes of the mantle or the magnetic field generation in the outer core.

The CMB represents only one element in the Earth's structure. The ultimate goal is to combine all the available data and invert them for the aspherical structure of the entire Earth. However, because of the difference in size of the subsets of data and their sensitivity to different elements of the Earth's structure, it is possible in such a procedure to introduce undesirable trade-offs and obscure an otherwise clear picture. At the present stage when the modelling strategy is still developing it is desirable to isolate an individual region within the Earth, use the available information on the structure above it and select the data that are particularly sensitive to its properties.

Assuming that the large scale mantle structure is known with sufficient accuracy we perform an experiment that focuses primarily on the shape of the CMB. In the process of verification of the result, using an independent set of data, we must, however, address a different question: that of the presence of lateral heterogeneity in the outer core.

The approach

The datum most sensitive to the depth of a discontinuity is the travel time of a wave reflected from its surface. For the reflection from the top of a discontinuity at a radius r, the perturbation in travel time, δt, due to a change in its radius, δr, is

$$\delta t = -\frac{2\delta r}{r}(\eta^2 - p^2)^{1/2} \qquad (1)$$

where $\eta = r/v(r)$ and p is the ray parameter[10]. In principle, if the velocity $v(r)$ is known, an observation of the travel-time anomaly of a reflected phase can be immediately converted into an equivalent change of radius of a discontinuity.

Phases reflected from the CMB have designations PcP and ScS for the compressional and shear waves, respectively. In routine reporting procedures, the arrival times of PcP are more numerous and reliable because of the sharper onset of this phase, owing to lower attenuation.

With the known mantle structure, a well distributed set of observations of PcP travel times would be sufficient to retrieve the shape of the CMB. For a spherically symmetric Earth model this was the approach that led Jeffreys[11] to his estimate of the core radius of 3,473.1±2.5 km; not far off from the currently used value of 3,480 km[12]. However, the data may not be uniformly distributed, and (in addition) there are possible sources of systematic errors that might put any such result in serious doubt, rendering the result inconclusive. Standard analysis, based on the assumption that the scatter in the data is random and uncorrelated, is meaningless if the sources of systematic errors, such as earthquake location or mantle structure, cannot be quantified and removed.

In addition to phases reflected from the CMB, there are waves transmitted through it: these are the so called core phases. For the purpose of a study based on routine reports, the most important phases are PKP (the AB and BC branches) and PKIKP (the DF branch). The latter designation denotes waves that traversed the inner core, and because there are reasons to suspect that this region is also laterally heterogeneous, we shall not use PKIKP in this experiment. Figure 1 shows the ray path of PcP and PKP phases and the inset is a plot of travel times of the core phases.

For waves transmitted through a discontinuity, the expression for perturbation in travel time is

$$\delta t = -\frac{\delta r}{r}[(\eta_+^2 - p^2)^{1/2} - (\eta_-^2 - p^2)^{1/2}] \qquad (2)$$

where the subscripts + and − denote, respectively, the values of η on the top- and bottom-side of the discontinuity[10]. The correction (2) must be considered for both the entry and exit points.

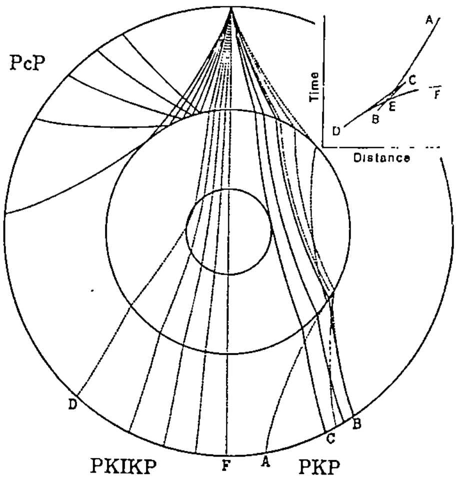


Fig. 1 Ray paths of waves reflected from core-mantle boundary, PcP, core phases bottoming in the outer core (branches BC and AB, broken line) and reaching the inner core: PKIKP (DF branch). Inset: diagram showing the travel times of the AB, BC and DF branches.

The velocity across the CMB changes abruptly: it drops from 13.7 km s^{-1} to 8 km s^{-1}; this makes the travel times of core phases relatively sensitive to the CMB radius. Considering a particularly simple case of vertical incidence ($p = 0$), a change in travel time of PcP is −0.146 s for a 1 km increase in the core radius; it is +0.051 s for PKP, but this must be counted twice, so a spherically symmetric increase in radius by 1 km would add 0.102 s to the travel time. Not much less than for PcP, but what is particularly important, the sign is opposite.

Adopting the approach of Dziewonski[13] of expanding the lateral heterogeneity in spherical harmonics, we write

$$\delta r(\theta, \phi) = \sum_{l=0}^{L}\sum_{m=0}^{l}[c_l^m \cos m\phi + s_l^m \sin m\phi]p_l^m(\cos\theta) \qquad (3)$$

where θ and ϕ are co-latitude and longitude and p_l^m are associated Legendre polynomials.

This may be substituted into equation (1) for PcP with the coordinates (θ_r, ϕ_r) corresponding to those of the reflection point. Such a system of equations of conditions can then be solved for the unknown coefficients c_l^m and s_l^m; L is the cutoff value determined empirically depending on the quantity, quality and distribution of the data. For PKP the procedure is similar, other than the equations of condition contain contributions from both the entry and exit points.

The experiment

The International Seismological Centre (ISC), an organization involving representatives from 40 countries, is compiling arrival times of seismic phases and other parameters that are contributed by over 1,000 globally distributed stations. These individual reports are associated with seismic events (earthquakes, explosions) and are published in monthly bulletins (BISC)[14]. Since 1964 the Bulletin information has also been available on magnetic tapes, which makes it feasible to retrieve and process a very large amount of data. The first such use of BISC, in the context of studying lateral heterogeneity of the Earth, was reported in 1975[15] and the use of this database is rapidly proliferating[3,4,6-8,13,16-20]. It is important to investigate the signal-to-noise ratio of this data set, because it may have a bearing on how models using this database should be parameterized.

The data for P, PcP, and PKP (BC branch) are extracted from the years from 1964 to 1982 for earthquakes that have at least 50 P-wave arrivals in the teleseismic distance range (25°–100°)

and not less than 5 arrivals in at least three azimuthal quadrants. The earthquakes are relocated using the most current travel-time table[13] (only events shallower than 50 km are used), azimuth independent station corrections[21] and corrections for ellipticity[10]. Data with an absolute value of a residual greater than 4 s are rejected. We define 'source regions' as having a constant area corresponding to 5° × 5° squares at the Equator and 'receiver regions'. All travel-time residuals for a particular phase connecting the same source-receiver pair are averaged to yield 'summary residuals'. The data set for P is by far the largest: 1,549,235 individual readings are averaged into 66,569 summary residuals; for PcP there were 26,125 individual readings combined into 5,182 summary data and for PKP_{BC} there were 82,403 observations combined into 4,160 summary residuals.

Depending on the seismic activity of a particular source region and the density of stations in a receiver region there is substantial variability in the number of individual observations, N, used to derive a summary datum. We sort all summary data into subsets according to N. We define the variance σ_N^2 as:

$$\sigma_N^2 = \frac{1}{M_N} \sum_{i=1}^{M_N} (\delta t_i^{(N)} - \bar{\delta t}^{(N)})^2 \qquad (4)$$

where M_N is the number of source receiver pairs with the count of N observations, $\delta t_i^{(N)}$ are the summary residuals and $\bar{\delta t}^{(N)}$ is the average residual of the Nth population.

Figure 2 shows the plots of σ_N^2 as a function of N for the phases: P *a*, PcP *b* and PKP_{BC} *c*. If all residuals were due to random errors, we would expect $\sigma_N^2 = \sigma_E^2/N$. On the other hand, if all residuals were caused by a deterministic process, such as the large wavelength lateral heterogeneity, we should have $\sigma_N^2 = \sigma_H^2 = \text{constant}$, as there is no reason to expect that the level of lateral heterogenity and number of connections for various source-receiver pairs are correlated. The results shown in Fig. 2 demonstrate that the actual σ_N^2 is a composite of the two models proposed above:

$$\bar{\sigma}_N^2 = \sigma_E^2/N + \sigma_H^2 \qquad (5)$$

Using linear regression we can estimate σ_E^2 and σ_H^2; the numerical results are shown in the figure. For the most numerous P-wave data set the r.m.s. residual of $(\sigma_N^2 - \bar{\sigma}_N^2)$ is only 0.025 s^2. The results illustrate that the BISC data set is noisy, with the variance, due to the effects that can be modelled deterministically, an order of magnitude smaller from that due to random noise (reading errors, lateral heterogeneity on a scale smaller than our 5° × 5° division).

The conclusion from this analysis is that the BISC database contains signals related to lateral heterogeneity but, because of the low signal-to-noise ratio, the model parameterization should be chosen such as to assure substantial spatial averaging which would lead to the improvement of the ratio. Otherwise, it is easy to obtain a model that is dominated by noise. The P-wave data set described above was inverted to yield model HELM46[22], similar to L02.56[13] but derived using a larger set of observations; both models are defined by 245 parameters. This can be compared with nearly 50,000 model parameters determined by Clayton and Comer[17], who used a similar data set. Because of the very uneven sampling of different elements of the Earth's volume by the BISC database, the optimum parameterization should consider a variable spatial resolution. Such a model has yet to be derived.

The data for PcP and PKP extracted from BISC according to the procedure described above are corrected for the effect of lateral heterogeneity predicted by model HELM46. Figure 3*a* shows the distribution of the reflection points of our PcP data set. It is nonuniform, with the coverage being poor in the Southern Hemisphere. For this reason we limit the expansion to degree and order 4; the coverage should be sufficient to determine perturbations with a resolving half-wavelength of 45°.

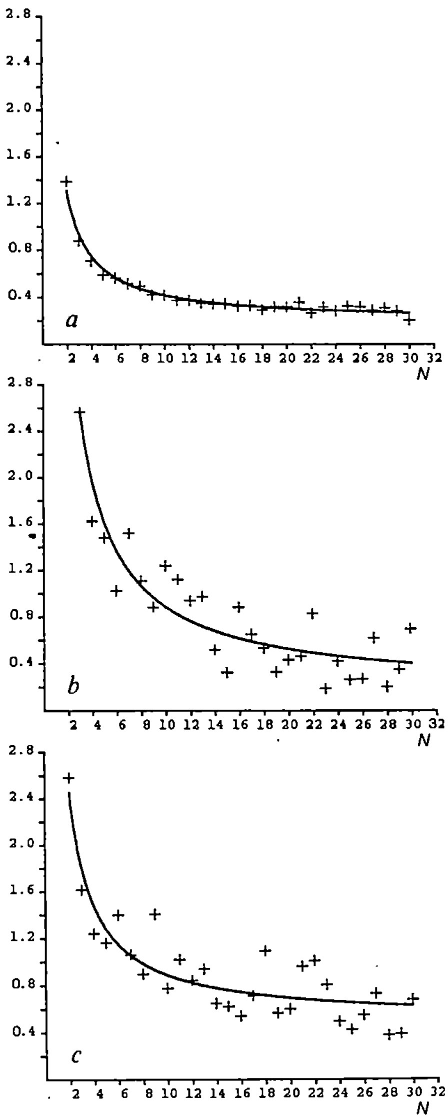


Fig. 2 Average variance of 'summary residuals' as a function of a number of individual observations, N, from which they were derived. *a*, P-waves; *b*, PcP and *c*, PKP_{BC}. Results of linear regression in search for σ_E^2 and σ_H^2 are shown for the individual data sets. In *a*, $\bar{\sigma}^2(N) = 2.0/N + 0.23$; *b*, $\bar{\sigma}^2(N) = 7.2/N + 0.16$; *c*, $\bar{\sigma}^2(N) = 3.9/N + 0.50$.

The map of the CMB obtained from inversion of the PcP data is shown in Fig. 3*b*. The largest feature is an area centred on Azores, elevated above the average by slightly more than 5 km; another high extends over the eastern Pacific; three lows, of the order of −4 km, are seen south of Japan and Colombia and north-east of New Zealand. The standard error analysis indicates that these variations are statistically significant. This, however, does not consider the possible errors introduced by mislocation of earthquakes or incomplete accounting for the mantle effects. The rim of the Pacific is depressed; this is a region of high velocities in model L02.56 of Dziewonski[13] (see map at a depth of 2,500 km). This would be consistent with the inference of Hager *et al.*[23], except for the features in the Pacific and the fact that the c_2^0 term is nearly zero.

The variance reduction of 3.5% appears misleadingly small. With the starting variance of 2.62 s^2, however, this corresponds to 0.092 s^2, or 57% of the total variance σ_H^2 which can be deterministically modelled (see Fig. 2*b*).

By itself the result, while interesting, might be considered inconclusive. Fortunately, there are other data (core phases) with resolving properties distinctly different from those of PcP. As Fig. 1*a* shows, the pattern of arrival times of core phases is relatively complex, particularly in the distance range from 144°

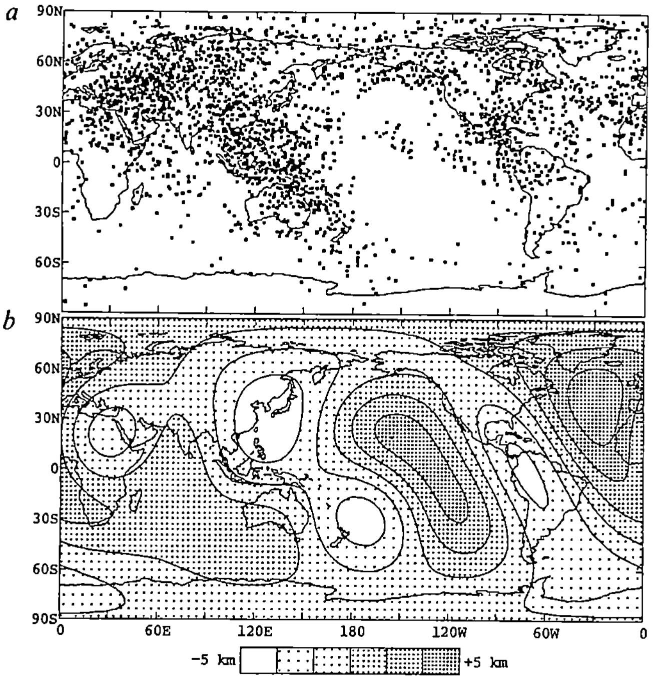


Fig. 3 *a*, Location of points of PcP reflection of 'summary data' used in this study. *b*, Topography of core-mantle boundary obtained by inversion of PcP residuals, corrected for lower mantle heterogeneity, for spherical harmonic coefficients up to degree and order 4.

to 150°-152°, where three branches, AB, BC and DF, are very close to each other. This picture, however, is misleading because it does not contain, in an explicit form, the amplitude information.

The retrograde AB branch has low amplitudes, is non-minimum-time and the signals are the Hilbert transforms of the original pulse, it is reported in BISC relatively infrequently and the data show large scatter. In addition, at distances beyond 160° the tunnelling effect of the mantle P-wave, nearly grazing the CMB, cannot be ignored[24]. On the other hand, the BC branch is relatively short (144°-154°), is prograde, has large $|d^2t/d\Delta^2|$ and, therefore, large amplitudes and, in effect, is frequently reported. The complication is that the BC branch is very close to the PKIKP (DF branch) between 145° and 151°. It was pointed out, however, by Anderssen and Cleary[25], that 85% of arrivals reported in the BISC (for this distance range) belong to the BC branch (see their Fig. 6), with the BC arrivals between 144° and 148° having amplitudes several times greater than DF pulses. Their finding is fully confirmed by our analysis, and we proceed on the assumption that all arrivals centred about the theoretical BC travel-time curve within ±4 seconds belong to the BC branch. The possible errors introduced by an occasional use of a PKIKP reading is readily offset by the volume and accuracy of the overall body of data. We show in Fig. 4 histograms of reported arrivals in one degree intervals centred on 145.5°, 150.5° and 153.5°. It is not expected that the DF amplitudes should change significantly between 145° and 153°; thus the dramatic difference in the total count must be attributed to the dominating influence of BC arrivals. At 153.5° all three branches are well separated in time.

The distribution of the points of the CMB crossing is shown in Fig. 5*a* and the resulting model of CMB topography in Fig. 5*b*. Even though the coverage is more even, the expansion is limited to degree 4, to make the direct comparison with the results obtained using the PcP more meaningful. The variance reduction of the PKP_{BC} data set is 10.5% of the original $1.82\ \mathrm{s}^2$, corresponding to $0.19\ \mathrm{s}^2$, or 38% of σ_H^2 (see Fig. 2*c*).

Discussion

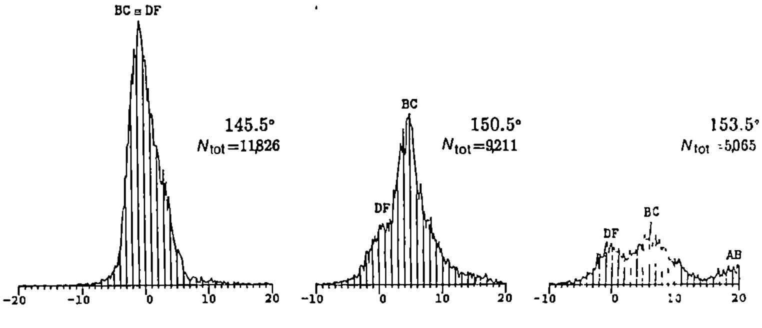
Even visual comparison of Figs 3*b* and 5*b* reveals significant similarity of the two maps in distribution and the sign of the perturbations in the shape of the CMB; the size is somewhat larger in Fig. 5*b*. The correlation coefficient is 0.701 on 23 degrees of freedom; the null hypothesis must be rejected at the confidence level above 99.99%. With the coefficients for degree 1 removed, which likely suffer from errors in earthquake location, the correlation coefficient increases to 0.744 on 20 degrees of freedom. Similarity between the two maps is greater in the Northern Hemisphere, where data coverage is better.

It was noted earlier that the kernels for PcP and PKP are of opposite sign. This means that the compatibility of the two results cannot be explained by a common source of systematic errors, such as the mislocation of sources in space and time or the effect of inaccuracies in our knowledge of the laterally heterogeneous mantle. These effects would result in errors of the same sign for PcP and PKP and, translated in terms of the CMB topography, should lead to maps with reversed polarity. This is not the case and we are led to the conclusion that the shape and magnitude of the anomalies are real.

Another important conclusion is that the level of large-scale lateral heterogeneity in the outer core is below the detectable level, as suggested in earlier studies involving phases PmKP, representing multiple internal reflections in the core[26]. It seems that, within acceptable errors, the effect inferred from PKP data is also seen in the map derived from PcP observations, and this phase does not enter the core. The PKP_{BC} reaches the bottom of the outer core, and if there was large-scale heterogeneity anywhere in this region, such as proposed by Ritzwoller *et al.*[5], it would be mapped as a perturbation in the shape of the CMB. Scientists studying the dynamics of the liquid core strongly resist[27] suggestions of lateral heterogeneities associated with density differences $|\delta\rho/\rho| > 10^{-5}$. It now seems that this view is supported by seismological evidence.

Creager and Jordan[6] studied travel-time anomalies of PKIKP and PKP_{AB} using BISC as the data source and projected the residuals, corrected for the mantle structure[13], onto a region in the vicinity of the CMB. They considered three hypotheses with regard to the source of anomalies: (1) D″ region above the CMB; they reject this hypothesis and our conclusion is the same; (2) perturbations in the radius of CMB and (3) a thin, highly heterogeneous layer under the CMB. They prefer the latter

Fig. 4 Histograms of PKP arrival times in 1° cells as reported in BISC[14]. The histograms demonstrate that the preponderance of the arrival times reported in BISC in the distance range from 144° to 154° belong to the BC branch.



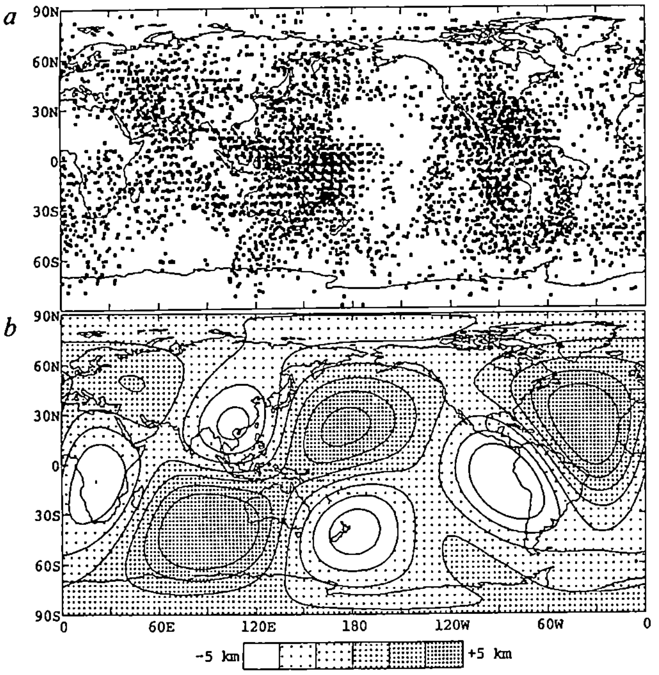


Fig. 5 *a*, Location of points of crossing of the CMB by rays corresponding to the 'summary data' PKP_{BC} used in this study. *b*, Topography of CMB obtained by inversion of PKP_{BC} residuals corrected for lower mantle heterogeneity.

hypothesis, even though their data cannot distinguish between (2) and (3). Their reason for rejection of (2) was the large range of CMB undulations, yet for the PKP_{AB} subset the range of the topography is similar to that proposed here and reported by Gudmundsson *et al.*[7]. The agreement between the results obtained in our report using PcP and PKP_{BC} indicates that (3) should be rejected as the only cause of the travel-time anomalies entering the core. However, the existence of a thin, homogeneous layer below the CMB with properties different (higher viscosity) from that of the bulk of the outer core should be given further consideration. There is an indication from observations of the offset between the figure axis of the earth and the rotational axis of the mantle that the 'astronomical' fluid-solid boundary is smoother than the 'seismic' CMB[28]. If such a layer was also characterized by a lower P-velocity, this could explain the difference between the amplitude of topography in Figs 3*b* and 5*b*. An increase in kernel (2) due to an increase in η_- would lead to a smaller topography in Fig. 5*b*.

Table 1 Spherical harmonic coefficients of the topography of the core-mantle boundary (CMB)

l	m	c_l^m	σ	s_l^m	σ
0	0	−2.50	0.20		
1	0	0.34	0.25		
	1	0.07	0.20	−0.03	0.22
2	0	0.14	0.24		
	1	0.55	0.19	−1.02	0.23
	2	0.29	0.20	0.00	0.20
3	0	−0.26	0.21		
	1	0.30	0.21	−0.04	0.21
	2	1.15	0.20	−0.31	0.17
	3	−0.11	0.20	−1.25	0.19
4	0	−0.11	0.22		
	1	0.22	0.21	0.36	0.20
	2	−0.44	0.22	0.12	0.19
	3	−0.66	0.21	−0.04	0.20
	4	−0.23	0.20	−0.68	0.20

To obtain relief in km, use equation (3); associated Legendre polynomials p_l^m are normalized according to equation (19) of Dziewonski[13].

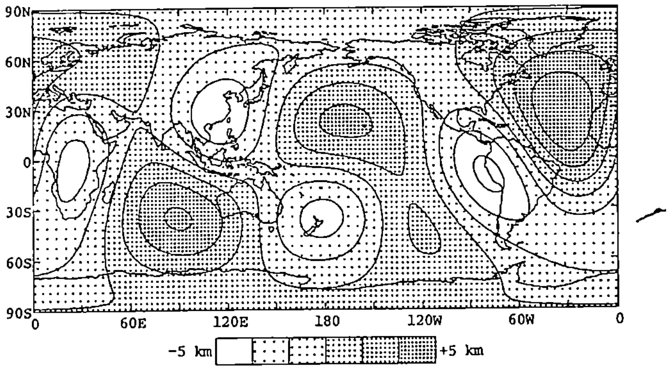


Fig. 6 Topography of CMB obtained by inversion of the combined PcP and PKP_{BC} data set. Spherical harmonic expansion is limited to degree and order 4.

While in some regions the pattern of anomalies of Creager and Jordan is similar to ours, there are also discrepancies. These could have resulted from differences in procedures applied in early stages of data reduction (earthquakes were not relocated in their study, for example).

In view of the compatibility of our results obtained separately from PcP and PKP data, these two sets can be merged and inverted again. The resulting map is shown in Fig. 6, and the coefficients of the spherical harmonic expansion are given in Table 1. They represent our best current estimate of the shape of the CMB. In the future, it may undergo some modification when PKIKP data are included in inversion, but a model of a laterally heterogeneous inner core must be obtained first. The spherically symmetric term implies a correction of −2.5 km with respect to PREM[12]; separate inversions of the PcP and PKP subsets of data yielded similar values. This leads to an estimate of the core radius of 3,477.4±0.2 km for the spherically symmetric model, a value very close to 3,477 km obtained by Taggart and Engdahl[29].

The joint inversion does not degrade significantly the fit to the two data sets previously considered separately: the percentage variance reduction for PcP changes from 3.5%, achieved with the model of Fig. 3, to 3.1% with the model of Fig. 6. For PKP_{BC} the value changes from 10.5% to 9.9%.

Hager *et al.*[23] calculated the perturbation in the CMB due to the dynamic load of sinking or rising density anomalies in the mantle; these density anomalies were inferred from aspherical velocity models by assuming the proportionality between density and velocity anomalies. The map in Fig. 6 does not compare favourably with the predictions of Hager *et al.* The flattening of the CMB departs insignificantly from that predicted from the assumption of hydrostatic equilibrium. This is in good agreement with Gwinn *et al.*[30] who inferred a departure of 490±110 m from very long baseline interferometry data. The correlation coefficient between the CMB topography and geoid for degrees 2 to 4 is −0.06. The differences between the results presented here and those of Hager *et al.* suggest that other static or dynamic effects must also be affecting the actual shape of the CMB.

Determination of the shape of the CMB may affect considerations related to the magnetic field of the Earth. Hide and Malin[31] proposed that 'bumps' at the CMB of size even less than reported here could significantly affect the pattern of flow in the fluid core. Even though his hypothesis has been questioned[32,33], it should be realized that we have retrieved only the gravest terms in the expansion. The observed scattering of 10 km wavelength waves at the CMB[34] indicates that irregularities may exist over a very wide range of lengths.

The derivation of the perturbations in the shape of the CMB should aid the analysis of PKIKP data in terms of the lateral heterogeneity of the inner core. The presence of aspherical perturbations in this region of the Earth has been reported in studies using travel times[3,4,8,35] as well as splitting of normal modes[1,9].

Finally, we would like to comment on the distribution of reported PcP arrivals shown in Fig. 3*a*. The difference in the density of reports cannot be explained by distribution of earthquakes and stations alone. It appears that, with some outstanding exceptions, seismic recordings are not read as diligently south of the Equator as to its north. Global studies, such as this one, are best conducted with a uniform distribution of data. We would like to appeal to the station operators and organizations such as the International Association of Seismology and Physics of the Earth Interior and the International Seismological Centre to encourage efforts that would lead to more frequent reporting of phases sampling in a relatively simple way the deep interior of the Earth. While it may take hundreds of millions of dollars to map the surface of Venus, the mapping of the more inaccessible interior of our planet could progress much more rapidly at negligible cost. Although there are good prospects that the Earth will be instrumented in the next few years by some 100 high quality broad-band digital stations[36-38], mostly designed for studies of waveforms, they will not replace the contribution that can be made by a careful analysis of seismograms at several thousand existing installations.

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A T-cell receptor γ/CD3 complex found on cloned functional lymphocytes

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Cloned blood lymphocytes that do not express the α- and β-chains of the T-cell receptor show MHC-unrestricted cytotoxicity. These cells carry the γ-protein, disulphide-linked either to another molecule or to itself, and associated with the CD3 complex. These observations may help to solve the mystery posed by the discovery of the γ gene.

T LYMPHOCYTES characteristically recognize foreign antigens in the context of molecules of the major histocompatibility complex (MHC). The molecular basis of this recognition has become amenable to investigation by virtue of the identification of the T-cell receptor (TCR) molecule, both at the protein[1-3] and the DNA[4-6] level. This molecule consists of two disulphide-linked chains, α and β, each containing a variable and a constant domain[7-9], encoded in different gene segments (V, D, J and C)[4-6,10] that join through rearrangement during T-cell ontogeny. The TCR heterodimer is physically linked at the cell surface with the CD3 (T3) protein complex[11-14], which consists of at least three different, non-polymorphic subunits[12,15]. Association with the CD3 complex is a prerequisite for surface expression of the TCR α and β chains[16,17]. The TCR $\alpha\beta$ molecule acts as the recognition unit determining T-cell specificity for antigen plus MHC molecules and the CD3 molecule may be important in subsequent signal transduction through the cell membrane, as reviewed in ref. 18.

Along with the TCR α and β genes, the putative TCR γ gene was identified; it rearranges specifically in T cells which are either cytotoxic[6,19] or helper cells[20]. The γ gene is organized in the same way as the α and β TCR genes[21], but its diversity may be more limited[22]. In man, at least ten variable (V), five joining (J), and two constant (C) gene segments have now been found (refs 22-28 and J.S. *et al.*, unpublished results). The TCR γ gene is rearranged and expressed relatively early in T-cell ontogeny, preceding α-gene rearrangement[29-31]. Recently, the protein product of the TCR γ gene has been identified[32] on peripheral blood lymphocytes (PBL) derived from immunodeficiency patients. The γ protein may also be expressed on $CD4^-8^-$

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Table 1 Characteristics of lymphocyte clones

Clone	Origin	Cell surface marker WT31	CD3	CD4	CD8	CD2	CD16	ADCC
AK 4	Normal* PB†	−	+	−	−	+	+	+
AK 615	Normal PB	−	+	−	−	+	+	+
AK 925	SCID PB	−	+	−	−	+	+	+
AK 119	Cancer PE†	−	+	−	−	+	−	−
AK 781	Normal* PB	+	+	−	−	+	−	−
CTL 20	Normal* PB	+	+	−	+	+	−	−
CAK 11	Normal PB	+	+	−	+	+	−	−
NK 77	Tγ-lymphocytosis PB	−	−	−	−	+	+	+

Cells were cultured by weekly stimulation with a feeder cell mixture consisting of irradiated EBV-transformed B cells and PBL as described previously[37]. Monoclonal antibodies used were OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8), OKT11 (anti-CD2) all obtained from Ortho, VD2 (anti-CD16) obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and WT31 (anti-TCR framework[38]), obtained from Dr W. Tax. Fluorescence was determined using a fluorescence-activated cell sorter (FACS II, Becton-Dickinson). Expression of the IgG-FcR as measured with the VD2 monoclonal antibody was always lower on CD3$^+$WT31$^-$ clones than on CD3$^-$ NK cells.

* Same individual; † PB, peripheral blood; PE, pleural effusion.

(T4$^-$T8$^-$) thymocytes[33]. Strikingly, the TCR γ-gene product occurs in association with the CD3 complex at the surface of T cells that do not express the α, β receptor[32,33]. So far, the role of the TCR γ protein in T-cell function has remained unclear.

We have previously described a small population (~2%) of peripheral CD3$^+$ T cells that are CD4$^-$8$^-$[34,35], a phenotype also found in the thymus[33,36]. We have now generated CD3$^+$4$^-$8$^-$ T cell clones from peripheral blood and describe here the phenotypic and functional characteristics of these cells; they express CD3 molecules, but most of them lack clone-specific TCR α and β molecules. Moreover, unlike most mature CD3$^+$4$^+$ or CD8$^+$ MHC-restricted cytotoxic T cells (CTL), but like CD3$^-$ natural killer (NK) cells, CD3$^+$4$^-$8$^-$ cloned lymphocytes have MHC non-restricted cytolytic capacity and can mediate antibody dependent cellular cytotoxicity (ADCC). The CD3 molecule is involved in the regulation of cytolysis. These clones lack productive α and β TCR gene rearrangements, but they do rearrange and express TCR γ genes. At the cell surface these CD3$^+$4$^+$8$^-$ T-cell clones express CD3-associated disulphide-linked heterodimers, comprising the γ protein and a non-γ protein that does not react with heterologous anti-γ serum. Our findings raise the possibility that this novel TCR is involved in MHC non-restricted cytolysis by T cells that use CD3 for signal transduction.

Cytotoxic function

Lymphocytes were cloned by limiting dilution[37] from PBL of healthy donors, of a patient with severe combined immunodeficiency (SCID), and from pleural effusion fluid of a patient with ovarian cancer. Five CD3$^+$4$^-$8$^-$ clones were studied in detail (Table 1). Strikingly, four out of five clones lacked the common epitope of the TCR heterodimer as defined by the monoclonal antibody WT31 (ref. 38), but did express the CD3 antigen. Indeed, 65% of CD4$^-$8$^-$ FACS-selected PBL were found to be CD3$^+$, and only 10% of those reacted with WT31 antibody.

CD3$^+$4$^-$8$^-$ cells freshly isolated from peripheral blood do not show substantial lytic activity towards K562 target cells[39], but after cloning and expansion in feeder cell mixture[37] they acquired cytolytic activity against a variety of tumour cells. The target cell spectra of CD3$^+$4$^-$8$^-$WT31$^-$ clones AK 615, 4 and 119 are given and compared with those of: (1) a CD3$^+$4$^-$8$^-$WT31$^+$ clone AK781; (2) a CD3$^+$4$^-$8$^+$WT31$^+$ CTL clone CAK 11, which interacts with class I MHC; (3) a CD3$^-$4$^-$8$^-$WT31$^-$ NK cell-derived clone NK 77 (Fig. 1). At effector to target cell (E:T) ratios of 3:1 and 27:1 the CD3$^+$WT31$^+$ clones AK 781 and CAK 11 showed only limited MHC non-restricted cytolysis, as observed earlier[40]. In contrast, the NK cell-derived clone NK 77 efficiently lysed K562, Daudi, monocytic and T-cell lines and a small cell lung carcinoma line (GLC1) at both E:T ratios. The CD3$^+$4$^-$8$^-$ WT31$^-$ clones AK 615, 4 and 119 lysed a variety of tumour cells at the E:T ratio 27:1, but Daudi cells were not lysed by AK 119. At the E:T ratio 3:1, the CD3$^+$WT31$^-$ clones showed a more selective and individual target cell spectrum.

As summarized in Table 1 and detailed elsewhere[41] three out of four CD3$^+$4$^-$8$^-$WT31$^-$ cloned cells also showed ADCC, a characteristic of NK cells expressing receptors for the Fc portion of immunoglobulin G(IgG-FcR; CD16). This finding functionally confirms the reactivity of the CD3$^+$WT31$^-$ clones with monoclonal antibodies directed against the IgG-FcR (Table 1).

CD3 regulates cytolytic activity

The anti-CD3 monoclonal antibody WT32 inhibited the lytic activity of CD3$^+$WT31$^-$ clones AK 4 and 615 against target cells Daudi and GLC1, but induced cytoxicity of clones AK 4, 615 and 119 provided target cells were used that express FcR for the IgG$_{2a}$ monoclonal antibody WT32 (U937 and P815; Table 2). This antibody did not induce cytolysis by clone AK 119 of Daudi, which does not express FcR for IgG$_{2a}$. Induction of lysis has previously been described for MHC class I- or II-specific T-cell clones[42-44].

Thus, we have demonstrated a unique category of CD3$^+$4$^-$8$^-$WT31$^-$ peripheral T cells. These cells share features with mature T cells in that they express the CD3 antigen, which is involved in the regulation of their lytic activity; however, they lack surface expression of the TCR αβ heterodimer. On the other hand, the clones also share features with CD3$^-$ NK cells, in that they display MHC non-restricted lysis, bear IgG-FcR and can mediate ADCC. Moreover, the clones also respond to the addition of recombinant interleukin-2 and β-interferon with augmented lytic activity[41].

Table 2 Anti-CD3 monoclonal antibody modulates the cytotoxicity of CD3$^+$4$^-$8$^-$WT31$^-$ clones

Clone	WT32*	% Specific ^{51}Cr release from target cell Daudi	GLC1	U937	P815
AK 4	−	44†	15	25	0
	+	16	4	45	62
AK 615	−	55	24	—	—
	+	13	10	—	—
AK 119	—	0	—	0	0
	+	0	—	55	31

Target cells Daudi, GLC1 and U937 are described in Fig. 1. P815 is a murine mastocytoma cell line.

* WT32 antibody (IgG$_{2a}$) detects the CD3 antigen[55] and was obtained from Dr W. Tax. Effector cells were incubated for 30 min with 1:100 dilution of WT32 ascites fluid before assay.

† Percentage ^{51}Cr-release in a 3-h cytotoxicity assay. Effector to target cell ratio was 3:1.

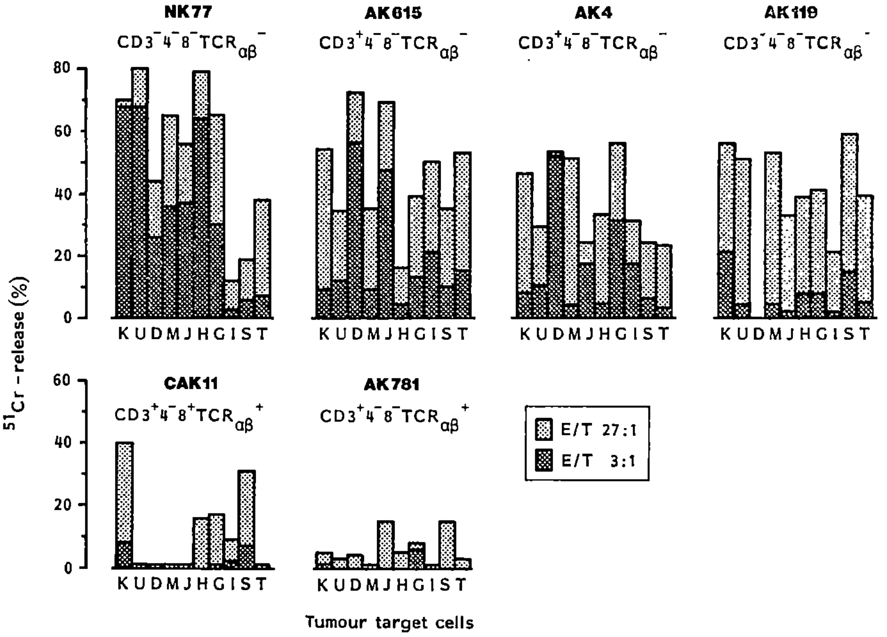

Fig. 1 MHC non-restricted cytolytic activity of CD3$^+$WT31$^-$, CD3$^+$WT31$^+$ and CD3$^-$WT31$^-$ clones. Cytolytic activity was determined in a 3-h ^{51}Cr-release assay in round-bottom microtitre plates. Characteristics of clones are outlined in Table 1. Tumour target cell lines: K, K562 (erythromyeloid); U, U937 (monocytic cell line); D, Daudi (B cell line); M, MOLT-4, J, Jurkat and H, HSB (T cell lines); G, GLC1 (from small cell lung carcinoma); I, IGR37 and S, SKMEL (melanoma derived); T, T24 (from bladder carcinoma). E:T, effector: target cell ratio.

TCR γ messenger RNA is expressed

We next investigated whether the TCR α,β heterodimer was expressed on the CD3$^+$WT31$^-$ T-cell clones in a form not detectable by antibody WT31. RNA was isolated from the CD3$^+$WT31$^-$ clones AK 4, 615 and 119, and, as positive controls, from the CD3$^+$ WT31$^+$ clone AK 781 and the T leukaemic cell lines Jurkat and HPB-ALL (Fig. 2). Mature α mRNA was present in Jurkat, HPB-ALL and clone AK 781, but was not found in clones AK 4, 119 and 615. No mature 1.3-kilobase (kb) β chain mRNA was found in clones AK 4, 119 and 615, but this message was expressed in Jurkat, HPB-ALL and clone AK 781. In clone AK 4 and (upon longer exposure of the autoradiogram) also in clones AK 119 and 615, a 1.0-kb β-chain message was detected, representing a defective transcript. We conclude that these CD3$^+$WT31$^-$ clones do not express TCR α and β chains.

As these cells lack TCR αβ expression but have a functional CD3 molecule they were candidates for the expression of the TCR γ protein. Clones AK 4, 119 and 615 do synthesize TCR γ mRNA, which is not produced in the TCR αβ$^+$ CD3$^+$4$^-$8$^-$WT31$^+$ clone AK 781 (Fig. 2). No rearrangement of the α chain gene was found in the CD3$^+$WT31$^-$ clones, although it cannot be excluded that a non-functional rearrangement had taken place but had remained undetected (unpublished results). A rearranged β-chain gene could be demonstrated in CD3$^+$ WT31$^-$ clone AK 4, but not in clones AK 925, 615 and 119 (Fig. 3*a* and data not shown). This rearrangement in clone AK 4 probably gives rise to the 1.0-kb mRNA observed in Fig. 2. As expected, rearrangements of both α and β chain genes were detected in the CD3$^+$4$^-$8$^-$WT31$^+$ clones AK 781 and CAK 11, but not in a CD3$^-$ NK-cell derived clone NK 77, consistent with previous reports[45,46].

Rearrangement of the TCR γ gene was studied by hybridization of a *Bam*HI digest with a genomic probe specific for the $J_{\gamma 1.3}$ gene segment (nomenclature and probe according to ref. 47). Only one γ-gene rearrangement was observed in DNA from CD3$^+$WT31$^-$ clones AK 615, 119 and 925 (Fig. 3*b*). The *Bam*HI digest of DNA from CD3$^+$WT31$^-$ clone AK 4 did not demonstrate an easily identifiable γ-gene rearrangement (not shown), but the CD3$^+$WT31$^+$ clones CTL 20 and AK 781 had undergone γ-gene rearrangements on both chromosomes. Thus, the CD3$^+$4$^-$8$^-$WT31$^-$ clones may have undergone significantly less gene rearrangement than the CD3$^+$ WT31$^+$ clones shown here, as well as leukaemic cells of such phenotype examined recently[23,26,48,49].

In conclusion, CD3$^+$WT31$^-$ clones: (1) may have unrearranged TCR α genes and do not express α mRNA; (2) may have rearranged TCR β genes but only to the extent that a non-functional, truncated mRNA can be made; (3) undergo γ gene rearrangement and produce mature γ mRNA.

CD3-associated disulphide-linked dimers

To determine whether the CD3 molecule present on the CD3$^+$WT31$^-$ clones was associated with molecules other than the TCR αβ heterodimer, the CD3$^+$WT31$^-$ clones AK 4, 615, 119 and 925 and the CD3$^+$WT31$^+$ clones CTL20 and AK 781 were surface-labelled with Na^{125}I. Immunoprecipitates were made using monoclonal anti-CD3 antibody and analysed by SDS-PAGE. Under both non-reducing and reducing conditions, the CD3 glycoproteins of relative molecular mass 19,000–21,000

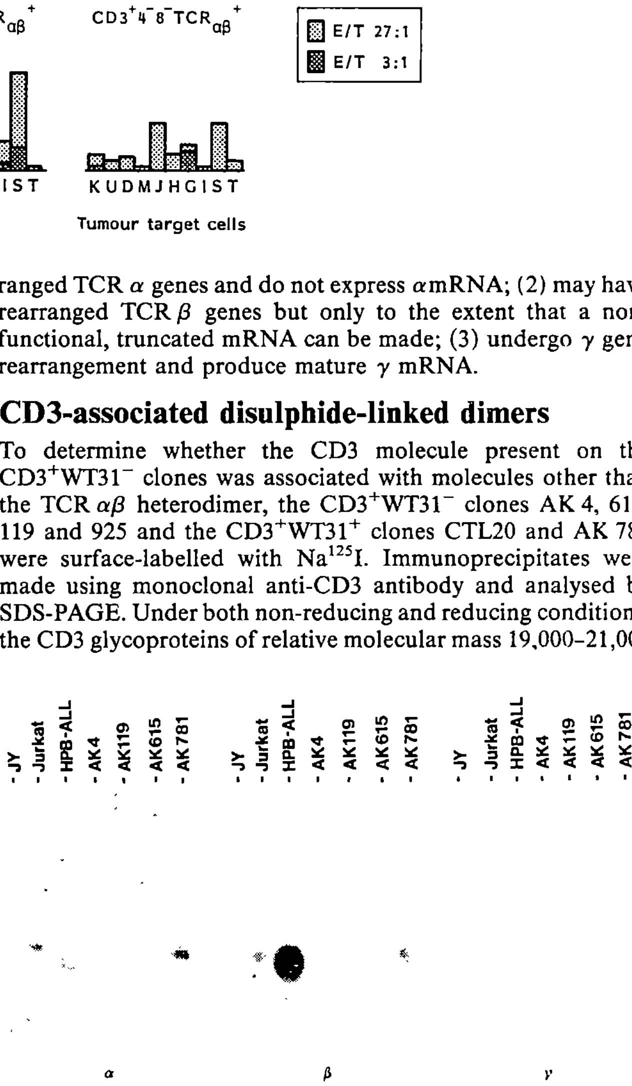

Fig. 2 Expression of TCR α,β and γ genes in CD3$^+$WT31 clones. Total cellular RNA was extracted according to the LiCl-urea method and electrophoresed at 10–20 μg per lane on a formaldehyde-containing 1.3% agarose gel. RNA was transferred to nitrocellulose and the same blot was probed three times with ^{32}P-labelled β, α and γ chain probes in the form of isolated inserts, in that order. Probes were removed by washing the blot for 5 min in 0.1 M NaOH, followed by neutralization in 10 mM Tris-HCl, *p*H 7.5, 3×SSC. Removal of the previously used probe was checked by autoradiography. The α-chain probe used was isolated from a complementary DNA library of HPB-MLT and kindly provided by Dr J. L. Strominger. The β-chain probe was HPB-β2, isolated from a cDNA library of HPB-ALL and a gift from Dr C. P. Terhorst. The γ chain probe was pTγ-1, isolated from an HPB-MLT cDNA library as described[28]. This method allows qualitative, but not quantitative measurement of mRNA expression.

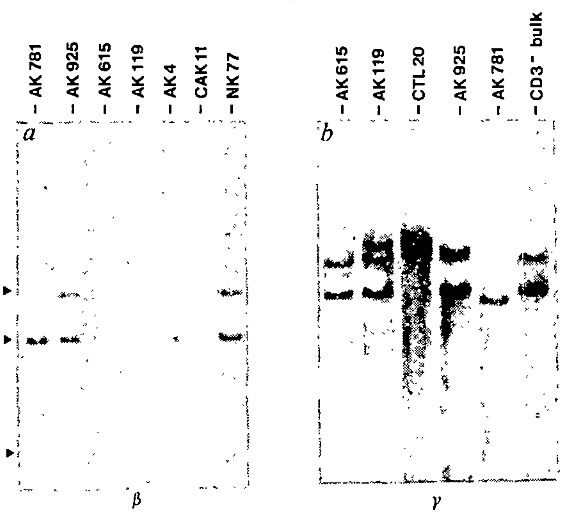

Fig. 3 Rearrangement of *a*, TCR β and *b*, TCR γ genes in CD3$^+$WT31$^-$ clones. High M_r DNA was extracted according to established procedures, digested to completion with the restriction endonucleases *Hin*dIII (β) or *Bam*HI (γ), electrophoresed on 0.6% agarose gels, and transferred to nitrocellulose sheets. Hybridization was performed at 42 °C in buffer containing formamide and 10% w/v dextran sulphate. Filters were washed at 55 °C, reducing the salt concentration of the wash buffer by step from 3 × SSC to 0.1 × SSC, at 0.1% SDS. The human TCR β chain probe used was an *Eco*RI-*Hin*dIII 2-kb genomic DNA fragment containing the $C_{\beta 2}$ constant region gene segment. This probe was a gift from Dr T. H. Rabbitts. The TCR γ probe was a genomic *Eco*RI-*Hin*dIII 0.8 kb fragment containing $J_{\gamma 1.3}$ (ref. 27). Arrows indicate germline configuration of β and γ genes.

(M_r 19-21K) (CD3δ) and 22-25K (CD3γ) can be seen, resolving as diffuse bands because of variations in the extent of glycosylation[12] (Fig. 4*a*-*d*). Under non-reducing conditions (Fig. 4*a*, *b*) an additional band was found of M_r 80K in the CD3$^+$WT31$^+$ clones CTL 20 and AK 781, of 70K in CD3$^+$WT31$^-$ clone AK 119 and 75K in CD3$^+$WT31$^-$ clones AK 4, 615 and 925. In CD3$^+$WT31$^+$ clones this 80K band is the disulphide-linked TCR α and β dimer[1-3]. Under reducing conditions (Fig. 4*c*, *d*), in CTL 20 and AK 781 the TCR α and β subunits are resolved independently, with M_r 46K and 36K and 44K and 39K respectively. In the CD3$^+$WT31$^-$ clones two prominent bands were found under reducing conditions with M_r 43K (1) and 40K (2) in clones AK 4, 615 and 925 and M_r 40K (2) and 36K (3) in clone AK 119. A third band with M_r 36K was also present in samples derived from clones AK 4, 615 and 925, but its intensity varied between experiments (compare experiments I and II). Given the M_r of the bands found under reducing conditions, the molecules of 75K and 70K seen under non-reducing conditions on clones AK 4, 615, 925 and AK 119 respectively must represent disulphide-linked dimers. In clones AK 4, 615 and 925 only the 43K and the 40K molecules may be required for dimer formation, as the dimer is also present when the 36K band is not detectable (see experiment I). In clone AK 119 the dimer could be composed of the 40K and 36K subunits.

The dimers contain the TCR γ chain

A rabbit antiserum raised against amino acids 137-157 of the TCR γ sequence[28,32] was used to determine whether the TCR γ gene product participated in the dimeric CD3-associated molecule on the CD3$^+$WT31$^-$ clones. Cell lysates were mildly denatured before immunoprecipitation, which allows detection of the γ protein, but also disrupts the interactions between CD3 and the γ components[32]. Figure 4*d* shows that the anti-Cγ serum precipitated two bands with M_r 40K and 36K from the four CD3$^+$WT31$^-$ clones. In contrast, no specific bands were isolated from the CD3$^+$WT31$^+$ $\alpha\beta^+$ clone AK 781. This demonstrates that the protein product of the TCR γ gene is synthesized in the CD3$^+$WT31$^-$ clones and strongly suggests that the 40K and 36K subunits associated with CD3 (Fig. 4*a*-*d*) represent the TCR γ proteins.

To prove this point, anti-CD3 immunoprecipitates were made from clones AK 781, 4 and 119 and treated so as to dissociate the protein complex from the antibody and partly denature its components[32]. Immunoprecipitation was then performed using anti-C$_\gamma$ serum. Figure 4*e* shows that the 40K and 36K components previously associated with CD3 were now selectively isolated, whereas no material was precipitated from an anti-CD3 sample derived from CD3$^+$WT31$^+$ clone AK 781. This shows that the 40K and 36K CD3-associated subunits bear TCR γ determinants. But the anti-Cγ serum did not identify a 43K protein in the CD3$^+$WT31$^-$ clones, although a molecule of this M_r was found to be associated with CD3 in clones AK 4, 615 and 925. Thus in clones AK 4, 615 and 925 the dimers are composed of a γ chain and a 43K non-γ chain. In clone AK 119, the dimer might be composed of two γ chains of 40K and 36K or, alternatively, of a γ chain and another component similar to the 43K subunit found in the other clones, which remained undetected for unknown reasons.

Relationship between 40K and 36K TCR γ chains

The 40K and 36K γ chains were further investigated by two-dimensional gel electrophoresis (isoelectric focusing followed by SDS-PAGE). Anti-CD3 precipitates made from CD3$^+$WT31$^-$ clone AK 119 and from CD3$^+$WT31$^+$ clone AK 781 as a control, were analysed before and after treatment with endoglycosidase F (endo F), which cleaves both high mannose and complex *N*-linked carbohydrates[50]. Before endo F treatment, TCR α and β chains isolated from clone AK 781 (M_r 44K and 39K) as well as CD3 components (M_r 19-25K) were heterogeneous in charge, due to the presence of sialic acids[12]. After deglycosylation the chains were virtually homogeneous in charge, the α chain being acidic and the β chain more basic. The CD3-associated 40 and 36K bands from clone AK 119 had very similar isoelectric focusing patterns, showing the close relationship between the two bands. After endo F treatment there was no difference in M_r between the two γ components. But a spot remained with an isoelectric point (pI) of ~8 that was also found before endo F treatment, and a slightly less basic spot that was probably the result of desialation of the molecules found in the two heterogeneous smears before endo F treatment.

Therefore, differential glycosylation causes the molecular weight difference found for the two γ chains in clone AK 119. However, the relationship between the two spots remaining after endo F treatment is not clear.

Discussion

Brenner *et al.* have recently reported CD3$^+$4$^-$8$^-$ or 4$^-$8$^+$ cells derived from PBL of immunodeficiency patients that lack the $\alpha\beta$TCR and express a CD3-associated TCR γ protein[32]. Bank *et al.* described a cloned cell line derived from CD3$^+$4$^-$8$^-$ thymocytes, which may express a CD3-associated TCR γ chain[33]. These cells showed no lytic activity unless treated with anti-CD3 monoclonal antibody.

We reported that a small population (~2%) of PBL obtained from normal donors shows the CD3$^+$4$^-$8$^-$ phenotype[34,35]. Most of these cells do not react with the anti-$\alpha\beta$ TCR monoclonal antibody WT31. Clones of this CD3$^+$4$^-$8$^-$WT31$^-$ phenotype derived from PBL of normal donors, of a SCID patient and from pleural effusion of a cancer patient were found to be functionally active: they can exert MHC non-restricted cytolysis and ADCC. These clones also express CD3-associated TCR γ chains. Clones of CD3$^+$WT31$^-$ phenotype have also been isolated from fetal PBL, but the nature of their receptor is as yet unknown[51].

The question is how these TCR γ-expressing CD3$^+$4$^-$8$^-$ cells fit into T cell differentiation pathways. Fetal thymocytes which

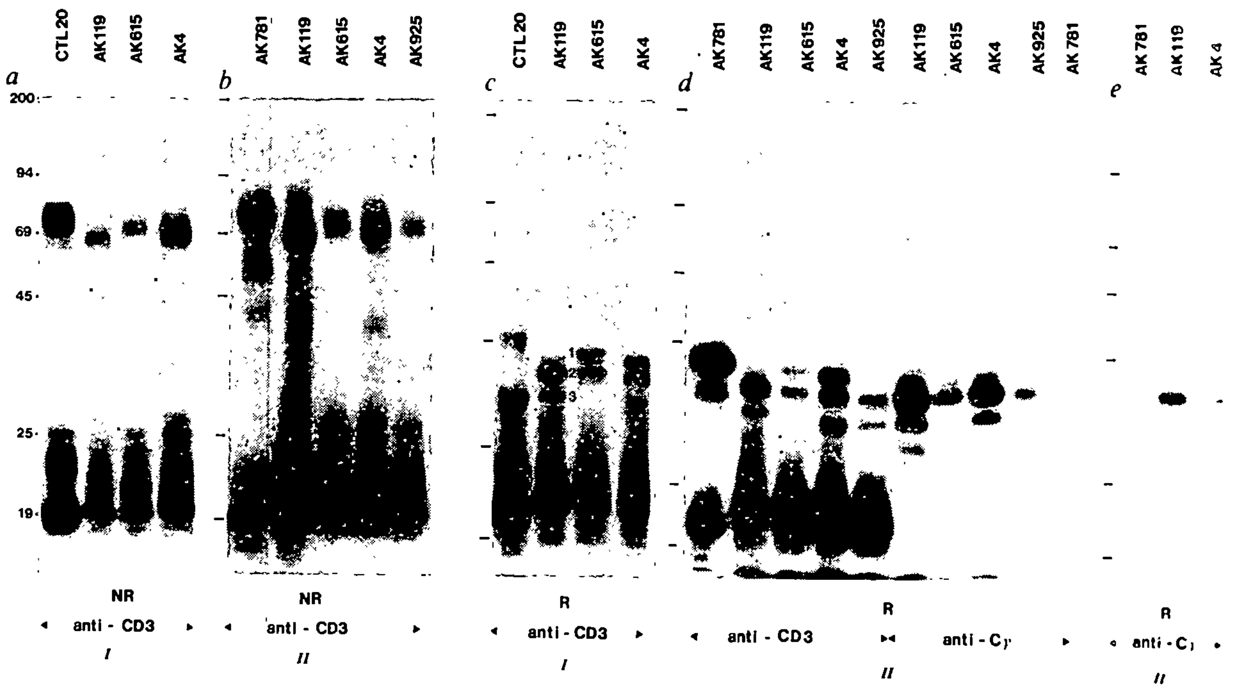

Fig. 4 SDS-PAGE of anti-CD3 and anti-Cγ immunoprecipitates made from ^{125}I-labelled CD3$^+$WT31$^-$ and CD3$^+$WT31$^+$ clones. About 20×10^6 cells were surface labelled with Na^{125}I using 1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril (Iodogen, Pierce Chemical Co.) as a catalyst. Cells were lysed in 0.01 M triethanolamine-HCl, *p*H 7.8, 0.15 M NaCl, 1 mM phenylmethylsulphonylfluoride (PMSF), and 0.02 mg ml^{-1} ovomucoid trypsin inhibitor (TI) (immunoprecipitation buffer, IPB), containing 1% digitonin[56] (Sigma) in the case of anti-CD3 immunoprecipitates, or 1% Nonidet P40 (NP40) for anti-C_γ immunoprecipitates. Lysates were precleared and anti-CD3 immunoprecipitation (*a*–*d*) was done with monoclonal anti-Leu-4 antibody coupled covalently to protein-A CL4B Sepharose beads with dimethylpilimidate-HCl. Beads were washed five times in IPB, 1% digitonin. For anti-Cγ immunoprecipitation (*d*), 1% SDS and 2 mM dithiothreitol (DTT) were added to the lysates, which were incubated for 5 min at 68 °C. Subsequently, samples were diluted with five volumes IPB, 1.5% NP40 and iodoacetamide was added to 20 mM. Lysates were precleared, anti-Cγ serum was added and immune complexes were recovered with protein-A Sepharose beads. Beads were washed five times in IPB, containing either 0.5% deoxycholate or 0.5% NP40. Samples were analysed on SDS-polyacrylamide gradient gels, either under non-reducing (NR, 1 mM iodoacetamide), or under reducing conditions (R, 5% v/v 2-mercapto-ethanol). I, samples derived from labelling I; II, samples derived from labelling II. For reprecipitation (*e*), anti-Leu-4 precipitates were incubated in 100 μl IPB containing 1% SDS and 2 mM DTT for 5 min at 68 °C. Beads were spun out and the supernatant was diluted to 500 μl with IPB, then 1.5% NP40, 20 mM iodoacetamide and 25 μg myoglobin were added and the supernatant was precleared once with normal mouse immunoglobulin-coated beads, after which anti-C_γ immunoprecipitation was performed as outlined above. 1, 43K band; 2, 40K band; 3, 36K band. Size markers are M_r in thousands.

ack TCR α mRNA can express CD3 δ and ε subunits[52]. This finding, combined with the fact that cells expressing significant levels of TCR γ message are present at an early stage of T-cell development in the thymus, suggests that certain early thymocytes may express γ/CD3 protein complexes at the cell surface. Indeed, such cells were reported by Bank *et al.*[33]. Therefore we postulate that the CD3$^+$4$^-$8$^-$WT31$^-$ cells found in peripheral blood undergo TCR γ gene rearrangement in the thymus, start expressing a γ/CD3 complex at the cell surface, and are subsequently exported as mature T cells.

In the CD3$^+$WT31$^-$ clones we observed rearrangement in only one allele of the TCR γ gene. Most human leukaemic T cell lines (48 out of 51) have gene rearrangement involving both γ gene alleles[23,26,48,49]. These rearrangements do not lead to γ protein expression in these cells but the TCR α and β chains are expressed. Therefore, we propose a model of T-cell differentiation in which the γ gene rearranges first on one chromosome and next on the other until a functional gene is formed leading to a TCR involving the γ protein. Such cells may represent a distinct mature T cell subset. Cells that fail to express γ chain protein then productively rearrange and express the TCR α and β genes, eventually producing a classic CD3-associated αβ TCR.

Here we show conclusively that CD3-associated disulphide-linked dimeric molecules that involve the TCR γ protein exist. Brenner *et al.* have reported a CD3-associated γ protein, non-covalently linked to a different, non-γ protein, which may form a putative second TCR[32]. Apparently, the γ protein is more versatile in its quaternary organization than the TCR α and β chains, which are invariably disulphide-linked. It is tempting to speculate that this difference in molecular organization is related to the differentiation stage and to the functional capabilities of the cells on which the TCR γ protein is expressed. We find clear indications of two different types of dimers: three clones express 40K and 36K chains which bear γ determinants, disulphide-linked to an additional 43K non-γ chain. One clone (AK 119) expresses only the 40 and 36K γ chains, which are probably disulphide-linked to one another, as the dimer on this clone has a slightly lower M_r. However, the possibility exists that the 43K non-γ chain has remained undetected. We suspect that the 40K and 36K chains bearing the γ epitopes are differentially processed protein products of the same gene; only one γ gene is rearranged in these clones. Moreover, one-dimensional peptide mapping according to Cleveland indicates extensive homology between these chains (results not shown). The nature of the 43K non-γ chain needs further study. The M_r of the CD3-associated chains differ from those reported by Brenner *et al.*[32], who found a γ chain of M_r 55K and a non-γ chain of 40K, but this may result from differences in glycosylation. Indeed, the human γ chain may harbour five, or possibly more (depending on *V* gene use) sites for *N*-linked carbohydrates[33]. It is interesting in this respect that Weiss *et al.*[53] have recently identified a CD3-associated chain that might be γ, with M_r 55–60K and a polypeptide backbone of 29K.

This is the first report suggesting a link between expression of the TCR γ protein and cytotoxic activity. At high E:T ratios the CD3$^+$WT31$^-$ clones display cytotoxic activity towards a wide range of target cells, but at low E:T ratios the lytic patterns

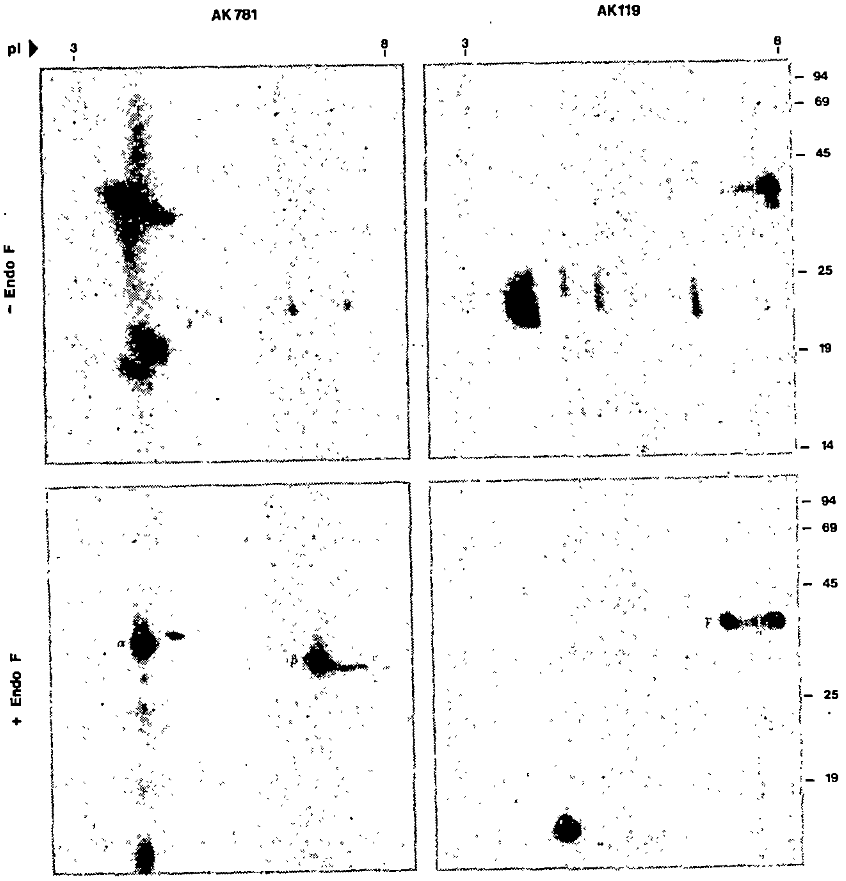

Fig. 5 Two-dimensional gel electrophoresis according to O'Farrell[57] of glycosylated and deglycosylated anti-CD3 immunoprecipitates derived from clones AK781 and 119. Samples were immunoprecipitated as described in Fig. 4. Deglycosylation with endo F[50] was performed as described[12]. Two-dimensional gel electrophoresis was performed using ampholytes (LKB, Bromma, Sweden) of *p*I 3.5-10, 4-6, 5-8 and 9-11 as 10:1:1:1. The second dimension was a 10-15% SDS polyacrylamide gel. Size markers, are M_r in thousands.

become more selective, in contrast to those of NK clones. The γ protein is associated with the CD3 antigen, which is analogous to the organization of the $\alpha\beta$ TCR. Cytolytic activity of both types of clones, bearing either an $\alpha\beta$ or γ TCR, is modulated by anti-CD3 antibodies, in contrast to that of CD3$^-$ NK cells. In view of its structural variability it would be anticipated that the γ protein on these cytolytic cells has a specific recognition function. Although a number of different tumour target cells were used here, the results obtained do not allow us to discriminate between the apparent MHC non-restricted cytotoxicity of CD3$^+$4$^-$8$^-$ γ expressing T cells and that of CD3$^-$ NK cells. More detailed analysis of the cytolytic patterns of the clones, using target cell panels of distinct histogenetic origins, may provide insight into their presumed specificities.

Considerable evidence is available to ascribe distinct functional roles to CD3$^-$NK cells and CD3$^+$TCR α,β^+ T cells in the immune response[54]. The CD3$^+$ TCR γ^+ T cells described here may constitute a separate T-cell population, with a distinct immune function.

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Two forms of the T-cell receptor γ protein found on peripheral blood cytotoxic T lymphocytes

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The T-cell receptor (TCR) γ polypeptide is expressed associated with CD3(T3) on the surface of normal human peripheral blood lymphocytes. These cells function as non-MHC-restricted cytotoxic T lymphocytes (CTL) and thus may play an important role in host immune defence. The TCR γ polypeptide occurs as a dimer in at least two molecular forms based on the absence or presence of disulphide linkage. These forms use TCR γ polypeptides with strikingly different peptide backbone sizes.

In addition to the TCR α and β genes, a third gene, γ, that rearranges in T cells exists in mouse[1-3] and in man[4,5]. This gene encodes a cell surface polypeptide of relative molecular mass 55,000 (M_r 55K) based on recognition by TCR γ-specific antipeptide sera[6]. The TCR γ polypeptide was found with another polypeptide, δ (40K), physically associated with the CD3(T3) glycoprotein on the cell surface[6]. TCR γδ lymphocytes were found to be $CD3^+4^-8^-$ (double negative) or $CD3^+4^-8^+$ in the peripheral blood of immunodeficiency patients and on a thymic-derived T cell clone[6,7].

The distribution and the function of cells that express the TCR γ product remain an enigma. At present, two lines of evidence suggest divergent roles for cells expressing the TCR γ polypeptide. Because TCR γ messenger RNA is expressed before expression of TCR β or TCR α mRNA during fetal thymic ontogeny in the mouse, a direct role in thymic differentiation of TCR αβ lymphocytes was suggested[8,9]. But the TCR γδ complex has been identified on human $CD3^+$ immunodeficiency patient lymphocytes that failed to react with framework monoclonal antibodies (mAb) against the TCR αβ complex (WT31 and βF1) and similarly failed to express mRNA encoded by the known TCR α and β genes[6]. Moreover, cells of the same phenotype, namely $WT31^-\beta F1^-CD3^+$ were found in normal human peripheral blood[6,10,11]. These results suggest that the second TCR might also occur on T lymphocytes that are part of the mature effector T cell repertoire.

Here we show that the TCR γ polypeptide occurs in normal human peripheral blood on $CD3^+$ non-major histocompatibility complex (MHC) restricted cytotoxic lymphocytes. Moreover, based upon the absence or presence of disulphide-linkage and peptide backbone size, CD3-associated TCR γ peptides are shown to exist in at least two molecular forms.

The non-disulphide-linked form

The immunodeficiency patient 2 (IDP2) cell line was sorted for $WT31^-$ cells and has been carried continuously *in vitro* for more than one year. It is homogeneously $WT31^-\beta F1^-CD2^+3^+$ ($T11^+T3^+$) and $CD4^-8^-$. Immunoprecipitations from lysates of surface-iodinated IDP2 lymphocytes using anti-CD3 mAb (under conditions that do not dissociate TCR subunits from CD3, see Fig. 1, legend) yielded two species (55K and 40K) in addition to the CD3 subunits (Fig. 1*a*). This result is identical

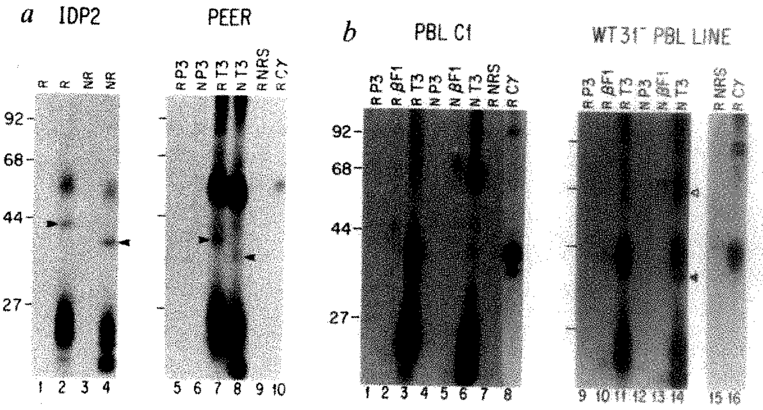

Fig. 1 Immunoprecipitations of TCR γ, δ and CD3 from a human tumour and peripheral blood lymphocyte lines. Immunoprecipitations from ^{125}I-labelled cell lysates were analysed by SDS-PAGE (10% acrylamide) under reducing (R) or nonreducing (N or NR) conditions. Size markers, M_r in thousands. *a*, TCR γ, δ and CD3 subunits on IDP2 and PEER cells. Immunoprecipitations were performed using 1 μg control mAb P3 (mAb secreted by the P3X63.Ag8 myeloma lanes 1, 3, 5 and 6); 1 μg UCHT1 (anti-CD3)[21] (lanes 2, 4, 7 and 8); 10 μl normal rabbit serum (NRS lane 9) and 10 μl anti-C_γ peptide sera (anti-TCR γ)[6] (lane 10). Arrows, positions of TCR δ subunits which change mobility under R and NR conditions. *b*, TCR γ, δ and CD3 subunits on peripheral blood T-cell clone, PBL C1, and the $WT31^-$PBL LINE. Immunoprecipitations were performed using control mAb P3 (lanes 1, 4, 9 and 12), 1 μg βF1 (anti-TCR β)[12] (lanes 2, 5, 10 and 13), NRS (lanes 7 and 15) and anti-C_γ peptide sera (lanes 8 and 16). Open arrow, disulphide-linked, βF1 unreactive CD3-associated species; solid arrow, non-disulphide-linked, CD3-associated material that displays increased SDS-PAGE mobility under nonreducing condition (like TCR δ in *a*).

Methods. Viable lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation and 2×10^7 cells were radio-iodinated by the lactoperoxidase technique as described[22]. Labelled cells were lysed in 5 ml of TBS (10 mM TRIS *p*H 8, 140 mM NaCl) with 0.3% 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulphonate (CHAPS), which preserves the TCR-CD3 association[23], containing 2 mM phenylmethylsulphonyl fluoride (PMSF) and 8 mM iodoacetamide (IAA). Immunoprecipitation was carried out using fixed *Staphylococcus aureus* Cowan I (SACI) as described[24], and the immune complexes were washed ×5 in TBS containing 0.1% Triton X-100 (TX-100). Reduced samples were boiled in 2 mM dithiothreitol (DTT) and all samples incubated for 10 min at 23 °C in 10 mM IAA before analysis by SDS-PAGE. Immunoprecipitations using anti-C_γ sera were performed on 1% TX-100 lysates that were dialysed to remove IAA and then denatured by the addition of $\frac{1}{10}$ vol of sodium dodecyl sulphate (SDS) containing 3 mM DTT with boiling for 3 min. After partial renaturation by the addition of 4 vols of 1.5% TX-100 in TBS containing 30 mM IAA, anti-C_γ sera or NRS were added and the imunoprecipitates were washed in TBS containing 0.5% TX-100, 0.5% deoxycholate, 0.05% SDS before analysis by SDS-PAGE. Rat anti-mouse κ chain-specific mAb 187.1 (5 μg) was added as a second antibody to provide protein A binding of IgG_1 mAb βF1, UCHT1 and P3[25].

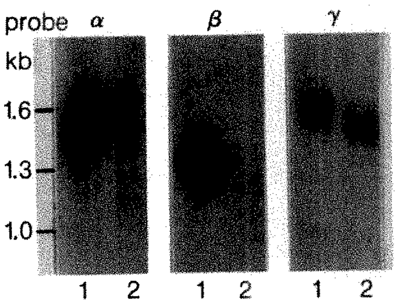

Fig. 2 Northern blot analysis of RNA isolated from PBL C1. Total RNA preparations from the T leukaemic cell line HPB-MLT (lane 1 for each probe) and PBL C1 (lane 2 for each probe) were analysed on Northern blots using TCR α, TCR β and TCR γ cDNA probes.

Methods. RNA preparation, agarose gel electrophoresis, transfer to nitrocellulose, hybridization with ^{32}P-labelled, nick-translated probes and autoradiography were as described[6]. Approximately 1.5 μg RNA was loaded per lane, probes were labelled to similar specific activity, and identical autoradiographical exposures are presented. RNA sizes were determined based on previously published lengths for TCR β and TCR γ transcripts[26,27].

to the one reported previously using chemical cross-linking. The 55K species was shown to react specifically with anti-C_γ and anti-V_γ peptide sera[6]. The 40K polypeptide was unreactive with these anti-γ peptide sera and is thus likely to represent a non TCR α, β or γ subunit, designated δ[6]. To determine if these subunits are covalently linked, like the TCR α and β subunits, the CD3 co-immunoprecipitated polypeptides were examined under reducing and nonreducing conditions. In striking contrast to the TCR αβ subunits, which exist in a heterodimeric disulphide-linked form under nonreducing conditions, the TCR γ and δ subunits on the IDP2 cell line are not covalently linked (Fig. 1*a*). A small increase in relative mobility on SDS-polyacrylamide gel electrophoresis (PAGE) under nonreducing conditions was observed for the diffuse, heavily glycosylated (see below)TCR γ, whereas a dramatic increase in mobility was observed for the δ subunit, suggesting the presence of one or more intrachain disulphide loops (compare species at arrows, lanes 2 and 4).

Weiss *et al.* suggested that the PEER cell line might express the TCR γ polypeptide as it lacked expression of TCR α mRNA yet expressed a CD3-associated 55-60K polypeptide[10]. On further examination, this cell line was found to lack reactivity with a mAb recognizing framework determinants on the TCR β chain, βF1 (Fig. 1*a*) and to express a strongly iodinated 55-60K polypeptide associated with a weakly iodinated 38K polypeptide. The 55-60K polypeptide was specifically immunoprecipitated with anti-C_γ peptide sera and thus appears to represent a further example of the TCR γ protein (Fig. 1*a*). The TCR γ and δ polypeptides on PEER were of similar size to those on the IDP2 cell line and similarly were not disulphide-linked. Like the δ subunit on IDP2 cells, the counterpart molecule on PEER underwent a marked shift in SDS-PAGE mobility when compared under reducing and nonreducing conditions (compare species at arrows, lanes 7 and 8). Thus the IDP2 and the PEER cell lines appear to express similar types of TCR γδ-CD3 complexes, in which the TCR γ and δ subunits are not covalently linked.

The disulphide-linked form

We wished to determine if this second TCR was also expressed as a component of the T cell population in normal peripheral blood. Two-colour cytofluorographic analysis comparing staining of human peripheral blood lymphocytes (PBL) with mAb βF1 and OKT3 (anti-CD3) showed a discrete population representing 2-5% of the CD3$^+$ PBL that appeared to be TCR αβ negative[6]. To examine this lymphocyte population, normal adult PBL were subjected to cytofluorographic cell sorting after staining with mAb WT31. Unstained PBL were isolated and propagated *in vitro* in IL-2-containing conditioned media receiving biweekly additions of irradiated autologous feeder cells and phytohaemagglutinin (PHA-P). The cell line derived, WT31$^-$PBL LINE, was cloned by limiting dilution with plating at 0.5 cell per well and the cloned cells were propagated as for the polyclonal cell line. Several such peripheral blood-derived T-cell clones were obtained, and PBL Clone 1 (PBL C1) was studied in detail. By cytofluorographic analysis, this clone was CD2$^+$3$^+$ but CD4$^-$8$^-$ and WT31$^-$. The expression of TCR α, β and γ mRNA from PBL C1 was determined by Northern blot analysis (Fig. 2). By comparison with the WT31$^+$βF1$^+$ T-cell tumour HPB-MLT, only very low levels of TCR α and β mRNA were detected. In contrast, abundant TCR γ mRNA was noted (Fig. 2, γ probe); interestingly, the TCR γ mRNA was slightly smaller than the 1.6-kilobase (kb) message found in HPB-MLT and in the TCR γ-expressing IDP2 cell line (Fig. 2 and ref. 6). Consistent with these observations, WT31 reactivity was not detected in cytofluorographic analysis (data not shown) and only scant levels of TCR α and β polypeptides were found by immunoprecipitation using mAb βF1 (Fig. 1*b*, lanes 2, 5). It is likely that the trace levels of TCR α and β protein detected in PBL C1 are accounted for by the 1-2% contamination with irradiated autologous feeder cells used in the propagation of this clone. In contrast, two abundant chains (40K and 36K) were observed associated with CD3 under reducing conditions in SDS-PAGE analysis (Fig. 1*b*, lane 3). Anti-C_γ sera immunoprecipitated both of these polypeptides from reduced and denatured PBL C1 lysates (Fig. 1*b*, lane 8).

To determine if these 40K and 36K TCR γ polypeptides were part of a disulphide-linked dimer, co-immunoprecipitations with anti-CD3 were examined under nonreducing conditions. A single band of M_r 70K was observed indicating that, unlike IDP2 and PEER cells, PBL C1 expresses a CD3-associated TCR γ gene product that is part of a disulphide-linked dimeric complex.

As a TCR γ partner, δ, was present on the non-disulphide-linked form of this receptor complex on IDP2 and PEER cells, we examined whether the disulphide-linked form of the receptor on PBL C1 was composed of a homo- or a heterodimer. Immunoprecipitates were analysed by two-dimensional gel electrophoresis (nonequilibrium *p*H gel electrophoresis (NEPHGE) followed by SDS-PAGE; Fig. 3*a*, *b*). Under reducing conditions, both the TCR γ species (40K and 36K) were found to have identical charges, and displayed heterogeneity typical for a sialylated glycoprotein. These characteristics are like those described previously for differentially glycosylated TCR α polypeptides having the same amino-acid backbone[12]. Thus, these species may represent differentially glycosylated forms of the same TCR γ peptide. This conclusion is supported by the results of metabolic pulse labelling (below) which reveal only a single precursor TCR γ species in PBL C1.

A disulphide-linked dimer composed of one or both of these TCR γ species should have a focusing position similar to either of the two components alone when analysed by NEPHGE or equilibrium isoelectric focusing (IEF). But a heterodimer composed of TCR γ and a distinct polypeptide might have a different charge and focusing position. The position of the disulphide-linked dimer was therefore examined by carrying out NEPHGE under nonreducing conditions, followed by SDS-PAGE under nonreducing conditions (Fig. 3*b*). Strikingly, the position of the disulphide-linked dimer was substantially more acidic than that of the TCR γ polypeptides examined under reducing conditions (compare the 40K and 36K species in Fig. 3*a* with the 70K species in Fig. 3*b*). This result suggests that the TCR γ species were covalently linked to a polypeptide of distinct NEPHGE mobility. Thus, although a TCR γ partner could not be directly visualized (either because it was inadequately labelled with ^{125}I or because it did not resolve in the focusing system used here),

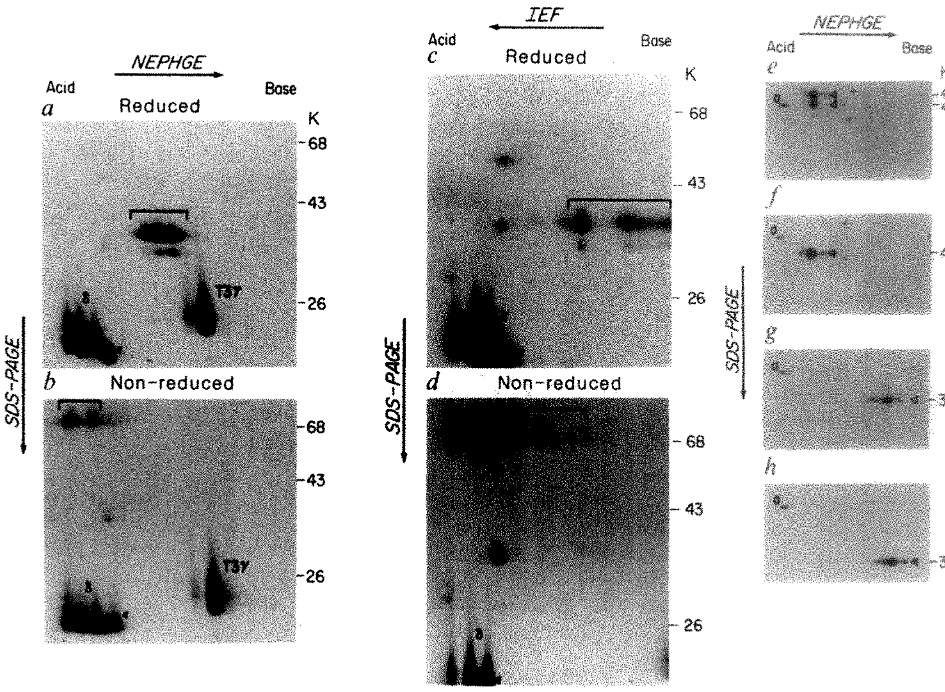

Fig. 3 Two-dimensional gel analysis of TCR γ polypeptides and precursors. *a–d*, Comparison of reduced (separated) and nonreduced (dimeric) CD3-associated polypeptides from PBL C1. ^{125}I-labelled PBL C1 cells were lysed in CHAPS and immunoprecipitated with anti-CD3 mAb. Two-dimensional gel electrophoresis was carried out under reducing conditions (*a*, *c*) or nonreducing conditions (*b*, *d*). The CD3 γ, δ and ε positions are labelled and focused to similar positions under both R and N conditions. After cleaving the disulphide bond, the CD3-associated polypeptides (40K and 36K) migrated to focusing positions close to CD3 γ under R conditions, but shifted to a more acidic position (close to CD3 δ) under N conditions (when both components of the dimer were present (68K)). T3γ represents CD3γ. Size markers, M_r. *e–h*, Analysis of glycosylated and nonglycosylated IDP2 and PBL C1 TCR γ peptide precursors. IDP2 and PBL C1 cells were pulse-labelled with ^{35}S-methionine, lysed under denaturing conditions and immunoprecipitated with anti-C_γ peptide sera. Immunoprecipitations were then either treated with endo-H or mock treated and analysed by two-dimensional gel electrophoresis. Glycosylated TCR γ peptides are denoted by open arrows, and nonglycosylated TCR γ peptides by solid arrows. Apparent M_rs were calculated from migration of standards used in panels *a* and *b* (not shown). A small amount of contaminating actin, denoted by a diamond in each panel, served as an internal marker. *e*, IDP2 TCR γ, mock incubated; *f*, IDP2 TCR γ, endo-H treated; *g*, PBL C1 TCR γ mock incubated; *h*, PBL C1 TCR γ endo-H treated.

Methods. *a–d*, After radioiodination with lactoperoxidase, lymphocytes were treated with 100 U of neuraminidase (Gibco) in phosphate-buffered saline (PBS), 1 mg ml^{-1} bovine serum albumin, 1 mg ml^{-1} glucose for 90 min at 23 °C, washed in PBS and solubilized in 0.3% CHAPS. Immunoprecipitates were prepared as in Fig. 1 and NEPHGE (charge separation) was carried out using *p*H 3.5–10 ampholines (LKB, Sweden), or IEF using *p*H 3.5–10, 4–6, 9–11 amphollines (2:1.5:1.5) followed by 10.5% SDS-PAGE gels for size separation as described[12]. NEPHGE was carried out (*a*, *b*) applying the iodinated sample at the acidic end; IEF (*c*, *d*) was carried out for 20 h at 400 V, applying the sample at the other (basic) end. Brackets, CD3-associated species. *e–h*, Cells (2×10^7) were preincubated for 1 h at 37 °C in 4 ml methionine-free RPMI 1640 supplemented with 10% fetal bovine serum. ^{35}S-methionine was added to 250 μCi ml^{-1}, and incubation was continued for 1 h at 37 °C. Cells were collected, washed and lysed in 0.4 ml of a boiling solution of 1% SDS, 10 mM Tris-HCl (*p*H 8.0), 0.1 mM PMSF and 10 mM IAA. Lysates were diluted with 1.6 ml of 2.5% Nonidet-P40, 1% gelatin, 10 mM Tris-HCl (*p*H 8), and 0.2 ml of 1 mg ml^{-1} DNase, 0.5 mg ml^{-1} RNase, 0.5 M Tris-HCl (*p*H 7), 50 mM $MgCl_2$, and incubated at 0 °C for 2–4 h. After centrifugation for 15 min at 12,000*g* to pellet insoluble debris, immunoprecipitations with anti-C_γ serum were performed using protein A Sepharose preincubated with 1% gelatin and washed as described[28]. Elution from the immunoabsorbent and treatment with endo-H (Miles Scientific) were as described[28]. Samples were analysed by two-dimensional gel electrophoresis employing NEPHGE in the first dimension and 10% SDS-PAGE in the second dimension, followed by fluorography[29].

the TCR γ polypeptide on PBL C1 appeared to be expressed as a part of a disulphide-linked heterodimer. Experiments using equilibrium IEF (rather than NEPHGE) confirmed this observation (Fig. 3*c*,*d*).

Different TCR γ peptide backbones

A further distinction between the disulphide-linked and nonlinked forms was the size of the mature TCR γ glycopeptide (55–60K on IDP2 and PEER versus 40K and 36K on PBL C1). To assess how much of this radical size difference is due to differential glycosylation and how much to different peptide backbones, TCR γ peptides were analysed in cells pulse-labelled with ^{35}S-methionine. After solubilization under denaturing and reducing conditions, the lysates were immunoprecipitated with anti-C_γ sera and examined by two-dimensional gel electrophoresis (Fig. 3*e–h*). Immunoprecipitates were either treated with endoglycosidase H (endo-H) to remove immature high-mannose glycans from pulse-labelled material, or were mock treated. Two TCR γ polypeptides (46K and 43K) of identical NEPHGE mobility were synthesized by the IDP2 cell line. Treatment with endo-H reduced both forms to a 40K form, suggesting that the 46K and 43K forms carried different numbers of carbohydrates, and that a single TCR γ polypeptide backbone (40K) was synthesized by IDP2 cells (Fig. 3*e*, *f*). In contrast, a more basic, 38K glycosylated form was synthesized by PBL C1, which after endo-H digestion displayed a nonglycosylated 31K peptide backbone (Fig. 3*g*, *h*). Thus, the TCR γ polypeptides on the non-disulphide-linked (IDP2) and the disulphide-linked (PBL C1) forms characterized here have radically different peptide backbone sizes (40K and 31K, respectively). The fact that the glycosylated TCR γ peptides observed by pulse-labelling are of different molecular weight from those found by cell surface iodination presumably results from the different types of carbohydrates they carry, namely high-mannose versus complex.

Two forms of TCR γ in peripheral blood

We next wished to determine if both a disulphide-linked and a noncovalently associated form occurred in normal adult peripheral blood. The polyclonal peripheral blood cell line (WT31$^-$PBL LINE) from which PBL C1 had been cloned was therefore studied in greater detail. WT31$^-$PBL LINE was homogeneously CD2$^+$3$^+$ and contained 95% WT31$^-$CD4$^-$8$^-$ with 5% contaminating WT31$^+$ cells. When examined by immunoprecipitation from iodinated, solubilized cells, weak but detectable reactivity with mAb βF1 was observed (Fig. 1*b*, lanes 10 reduced and 13 nonreduced), consistent with the expected 5% TCR αβ positive lymphocytes. In contrast, anti-CD3 mAb immunoprecipitated large amounts of both CD3 and associated polypeptides of 35–45K under reducing conditions (Fig. 1*b*, lane 11). To determine what fraction of these were disulphide-linked, the CD3 immunoprecipitate was examined under nonreducing conditions (Fig. 1*b*, lane 14). Less than half of the

CD3-associated polypeptides were disulphide-linked. This material included disulphide-linked TCR $\alpha\beta$ peptides located above the open arrow, lane 14 (size identified by the βF1 precipitate, lane 13) and disulphide-linked TCR γ peptides of smaller size (open arrow, lane 14). Strikingly, the majority of the CD3-associated species were not disulphide-linked and migrated with the same mobility under both reducing and nonreducing conditions. Notably, a fraction of these non-linked species displayed a marked increase in SDS-PAGE mobility under nonreducing conditions, similar to the TCR δ on the IDP2 and PEER cells (see Fig. 1*b*, lane 14, solid arrow). Reactivity with anti-C_γ sera confirmed that most of the labelled material associated with CD3 expressed on WT31$^-$PBL LINE was TCR γ gene product (lane 16).

Thus, the protein product of the TCR γ gene occurs on CD3$^+$ lymphocytes in adult peripheral blood in both disulphide-linked and unlinked molecular forms. Moreover, the non-disulphide-linked form of TCR γ may be further divided into 55-60K glycosylated (IDP2 and PEER) or 35-45K glycosylated (thymic T-cell clone CII[7] and WT31$^-$PBL LINE) species.

TCR γ rearrangements

TCR γ and β gene rearrangements were examined in T cells known to express the TCR γ polypeptide on their cell surfaces. Southern blot analyses were carried out using the 0.8-kb *Eco*RI-*Hin*dIII human $J_{\gamma1.3}$ probe (nomenclature according to Quertermous *et al.*[13]). This probe detects germline bands of 23 kb and 12 kb in a *Bam*HI digest of genomic DNA. The 23-kb band encompasses $C_{\gamma1}$, and the 12-kb band encodes $C_{\gamma2}$. Using this probe, IDP2, PBL C1 and PBL C2 (also derived from the WT31$^-$PBL LINE) showed rearrangements of the TCR γ gene (both PBL C1 and PBL C2 displayed an identical rearrangement; Fig. 4*a*).

Seven rearrangements in PBL using the $J_{\gamma1.3}$ probe and *Eco*RI-digested genomic DNA in Southern blot analyses have been detected[14]. Six (I, II, III, IV, VII and V) of these seven rearrangements are shown in PBL, fetal thymus, and newborn thymus genomic DNA (Fig. 4*b*; see arrows and rearrangement numbers). Four rearrangements (numbers I, II, VI and VII) either are not used by peripheral blood lymphocytes which express the TCR γ polypeptide or cells demonstrating them were lost under the propagation conditions used for the WT31$^-$ PBL LINE. Nevertheless, the WT31$^-$PBL LINE DNA revealed at least three of these rearrangements (III, IV and V) (Fig. 4*b*, lane 3) and these same rearrangements were used by IDP2, PBL C1 and PBL C2 (data not shown for the *Eco*RI digest) and all of these rearrangements are displayed in fetal thymus.

The TCR β gene was also rearranged in IDP2, PBL C1 and PBL C2 cells. The 1.1-kb *Eco*RI-*Hin*dIII $C_{\beta2}$ probe detects a germline band of 20 kb which encompasses both C_β constant regions in a *Bam*HI digest of genomic DNA[15]. One predominant TCR β rearrangement for IDP2 and two identical rearrangements for PBL C1 and PBL C2 were observed (Fig. 4*c*). It is assumed that these TCR β rearrangements are nonproductive based on the immunoprecipitations and Northern analyses for these cell lines. As both PBL C1 and PBL C2 have the same TCR γ and β rearrangements, they appear to be clonal and derived from the same cell within the WT31$^-$PBL LINE.

Non-MHC restricted cytotoxic lymphocytes

As TCR γ-expressing cells were found in adult peripheral blood, functional studies were carried out to determine whether they have effector capabilities. When IDP2 and PBL C1 were examined for their ability to lyse target cells in ^{51}Cr release assays, they proved to have spontaneous effector cytotoxic capability (Fig. 5). Although the IDP2 cell line did not lyse the majority of natural killer (NK) targets or PHA blasts of allogeneic PBL, they were selectively capable of lysing ^{51}Cr-labelled MOLT-4 cells (Fig. 5*a*, top). In two of six similar assays, weak lysis (10-15% ^{51}Cr release) of K562 targets was also observed. Lysis of MOLT-4 cells was not inhibited by a variety of mAbs directed against monomorphic MHC Class I (W6/32, anti-HLA-A, B, C, 4E and 131) or Class II (LB3.1 and anti-Leu 10) determinants (Fig. 5*b*), although we have previously found that these mAbs efficiently block killing by both MHC Class I and Class II allospecific CTL[16]. These data suggest that lysis of MOLT-4 cells was MHC class I and II independent. Only anti-CD3 mAb partially blocked the specific lysis of MOLT-4 cells (Fig. 5*b*). On the other hand, when triggered by prebinding of anti-CD3 mAb to IDP2, as has been previously reported for thymic-derived CII[7], ^{51}Cr-labelled target cells that express Fc receptors for IgG (for example, U937), were efficiently lysed (Fig. 5*a*, +anti-CD3). Such killing could be completely inhibited by aggregated human IgG, confirming that this CD3-mediated lysis occurred through a mechanism of enhanced conjugate formation via IgG Fc receptors (data not shown). The paradoxical augmentation of lysis by anti-CD3 mAb for some targets (U937) and the blocking of lysis for specifically recognized targets (MOLT-4) might result from the competing effects of triggering and increasing conjugate formation but sterically blocking antigen recognition via the TCR.

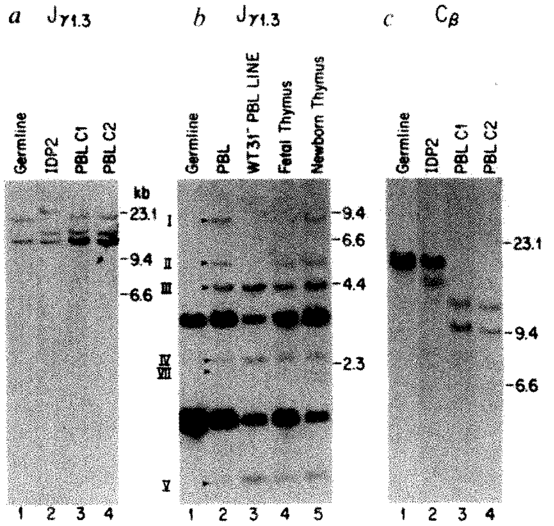

Fig. 4 Rearrangements of the γ and β genes in T cells expressing the TCR γ polypeptide. Genomic DNAs isolated from the IDP2 cell line, PBL C1, PBL C2, WT31$^-$PBL LINE, fetal thymus, newborn thymus, PBL and a B cell line (JY for germline) were examined in Southern blot analysis for TCR γ (*a*, *b*) and TCR β (*c*) gene rearrangements. Genomic DNAs were digested with *Bam*HI (*a*, *c*) or *Eco*RI (*b*), fractionated on agarose gels and transferred to nitrocellulose filters for hybridization with ^{32}P-labelled $J_{\gamma1.3}$ (*a*, *b*) or $C_{\beta2}$ probes (*c*). Arrows and roman numerals denote TCR γ rearrangements. Size markers in kb.

Methods. Genomic DNA was isolated as described[30], digested with endonuclease (*Bam*HI or *Eco*RI), size-fractionated on 0.7% agarose (*Bam*HI digests) or 0.9% agarose (*Eco*RI digest), and transferred to nitrocellulose as described[31]. Filters were hybridized to a nick-translated ^{32}P-labelled 0.8-kb *Hin*dIII-*Eco*RI $J_{\gamma1.3}$ probe (ref. 14 and unpublished) or a 1.1-kb *Eco*RI-*Hin*dIII $C_{\beta2}$ probe[15]. Filters were washed in 2 × SSC and 0.1% SDS followed by 0.2% × SSC and 0.1% SDS at 55 °C before autoradiography with intensifying screens.

PBL C1 proved a more efficient killer cell than IDP2. PBL C1 displayed spontaneous cytolytic activity against K562 cells (MHC Class I and II negative) showing nearly 50% specific ^{51}Cr release when examined at an effector:target (E:T) ratio of 20:1 (Fig. 5*c*, top). Moreover, PBL C1 also lysed MOLT-4 cells and to a lesser extent, CEM cells. No lysis of Daudi, U937, or either autologous or allogeneic PBL was detected. Triggering with anti-CD3 mAb induced PBL C1 to lyse the U937 cell line. Further, lysis of K562 was slightly augmented whereas that of MOLT-4 was partially inhibited (Fig. 5*c*). Taken together, the spontaneous cytolytic activity of IDP2 and PBL C1 on tumour

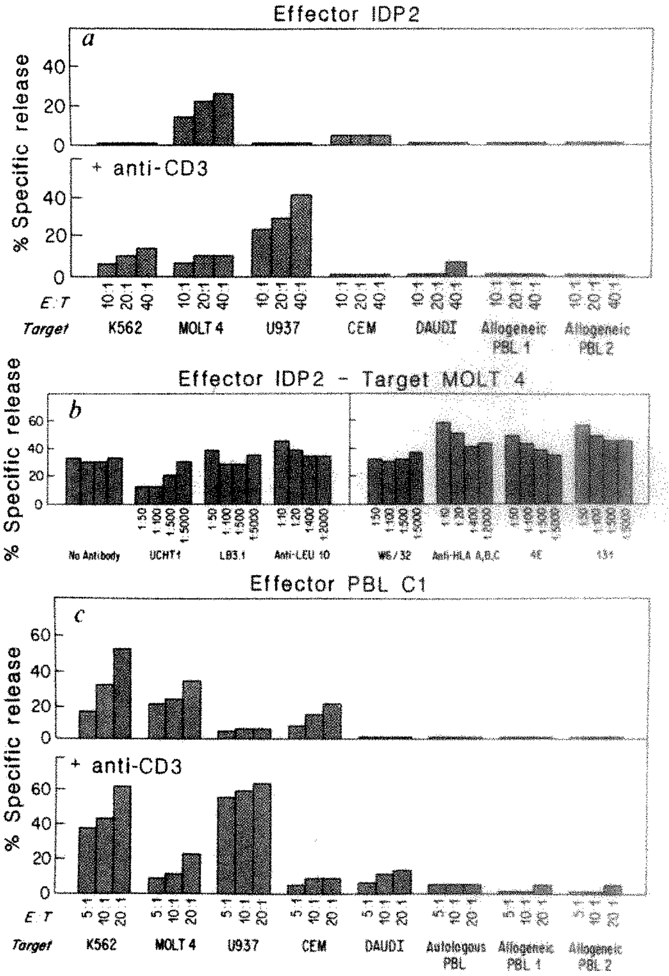

Fig. 5 Cytolysis by IDP2 and PBL C1 cells. *a*, *c*, IDP2 or PBL C1 effector cells were incubated (at effector:target (E:T) ratios indicated) with ^{51}Cr-labelled target cells K562 (erythroid line), U937 (monocytic line), MOLT-4, CEM (T leukaemic lines), Daudi (Burkitt's lymphoma line) or allogeneic or autologous PBL (3-day PHA blasts of human PBL). The % specific release of ^{51}Cr for each target is shown. The same assays were carried out after prebinding anti-CD3 mAb UCHT1 to the effector cells for 30 min at 0 °C (+ anti-CD3). *b*, Inhibition of IDP2 cytolysis of MOLT-4 target cells by various mAbs. IDP2 and ^{51}Cr-labelled MOLT-4 cells were incubated together at a 40:1 E:T ratio in the presence of various dilutions of anti-MHC Class I mAb W6/32 (anti-HLA-A, B, C monomorphic determinant)[32], anti-HLA-A, B, C (monomorphic determinant)[33], 4E (anti-HLA-B and C locus)[34], 131 (anti-HLA-A locus)[35] or anti-MHC Class II mAb LB3.1 (anti-DR specific)[36], anti-Leu 10 (anti-DQ specific)[37] or anti-CD3 mAb UCHT1. Higher dilutions were used for mAb available as ascites (W6/32, 4E, 131, LB3.1, and UCHT1) and lower dilutions were used for commercial mAb (anti-Leu 10) or culture supernatant (anti-HLA-A, B, C).

Methods. Cytolytic assays were performed in round-bottom 96-well tissue culture plates with ^{51}Cr-labelling, harvesting and calculation of % specific release as described[16]. For *a* and *c*, IDP2 or PBL C1 cells were either preincubated with UCHT1 (1:300 dilution) (+ anti-CD3) for 30 min at 0 °C, washed ×3 or mock incubated and placed together with labelled target cells. Anti-HLA Class I and Class II mAb or anti-CD3 mAb were placed in wells containing the ^{51}Cr-labelled MOLT 4 cells for 30 min at 0 °C, then IDP2 cells were added at a 40:1 E:T ratio. All samples were assayed in triplicate, each experiment was performed at least three times, and one representative experiment of each is shown.

argets such as K562 and MOLT-4 and the failure to block such ictivity by anti-MHC mAb suggests these TCR γ lymphocytes ire non-MHC class I- or class II-restricted CTL.

Discussion

TCR γ (55K) and δ (40K) have previously been identified as CD3-associated peptides on immunodeficiency patient cells. Here, these two subunits are shown to be noncovalently associated. Importantly, the TCR γ polypeptide is also shown to be part of a second TCR that occurs on normal adult peripheral blood lymphocytes. A T-cell clone isolated from peripheral blood (PBL C1), expresses two peptides of 40K and 36K that pecifically react with anti-C_γ peptide sera. In contrast to the DP2 cell line, these TCR γ polypeptides on PBL C1 are of maller size and are part of a disulphide-linked complex. This lisulphide-linked complex appears to be a heterodimer as the ocusing mobility of the dimer was drastically different from hat of the TCR γ polypeptide. The TCR γ polypeptides from he disulphide-linked and non-linked forms not only have lifferent glycosylated sizes (55–60K versus 36–40K) but also ave different peptide backbone sizes (40K versus 31K, respecively) as well as different mRNA transcript lengths. Moreover, analysis of a PBL-derived polyclonal population (WT31⁻PBL INE) which was composed predominantly of TCR γ lymhocytes revealed both disulphide-linked and non-linked TCR γ omplexes. Interestingly, the non-disulphide-linked TCR γ subnits observed on PBL were of smaller glycosylated size (35–5K) than the non-disulphide-linked TCR γ species on the IDP2 nd PEER cell lines (55–60K). Thus, TCR γ exists on the cell urface in at least two molecular forms (disulphide-linked and on-linked) and as two drastically different polypeptide sizes glycosylated 55–60K and 36–40K). Both TCR glycopeptide izes have been observed in non-disulphide-linked forms, whereas only the smaller size has been observed in the disulphide-linked form.

The TCR γ lymphocytes examined here exhibit non-MHC-restricted cytolytic activity and may be similar to other CD3⁺ NK-like cells whose T-cell receptors have not yet been definitively characterized[17–20]. As CTL they may participate in host immune surveillance against malignancy or infection. The specificity of lysis observed suggests the possibility of TCR γ mediated antigen-specific recognition of some but not all tumour targets. As anti-CD3 mAb could trigger nonspecific lysis of some target cells or alternatively block specific lysis of other targets, the CD3 molecule on these cells appears to be functional. This conclusion has been confirmed by the observation that anti-CD3 mAbs are capable of triggering abrupt changes in the levels of intracellular free calcium[38].

Based on the sequential expression of TCR γ, β and α mRNA during fetal thymic development, a developmental role for the TCR γ in the ontogeny of TCR αβ cells has been suggested[8,9]. The data reported here suggest that cells that have productively rearranged and expressed the TCR γ protein on their cell surfaces are mature cells found in peripheral blood. These cells use several of the TCR γ gene rearrangements that can be detected in fetal thymus. The relationship of TCR γ rearrangements in peripheral blood and thymus is consistent with the possibility that TCR γ cells pass through a stage of differentiation in the thymus. In this case, T cells that productively rearrange the TCR γ gene and express TCR γ at the cell surface may exit to the periphery as mature T cells and those that do not (either because TCR γ is nonproductively rearranged or because the TCR γ partner is not expressed) may then go on to rearrange and express the TCR αβ.

Use of a functional CD3 molecule and a TCR polypeptide encoded by a rearranging gene suggests that, like TCR αβ lymphocytes, TCR γ lymphocytes may be capable of antigen-specific recognition and clonal expansion. As these cytotoxic T cells are found in peripheral blood, lymphocytes expressing the TCR γ polypeptide must be considered as part of the mature repertoire of functional T cells.

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LETTERS TO NATURE

Low-frequency divergent X-ray variability in the Seyfert galaxy NGC4051

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The X-ray emission from NGC4051, a nearby low luminosity Type 1 Seyfert galaxy, exhibits variations that are both rapid and apparently quasi-periodic[1,2]. Many Seyfert galaxies are variable[3] but our knowledge of the form of variability has not advanced substantially since early well-resolved observations[4] and attempts at statistical description[5]. Here we report an uninterrupted 62-h observation of NGC4051 with the EXOSAT observatory, giving an order of magnitude improvement in the quality of the time series, allowing comparison with X-ray binaries. The observed power spectral density is roughly proportional to 1/*f*, similar to Cyg X-1, and characteristic of turbulent processes. No preferred timescale or luminosity gradient has been seen so far. If this behaviour holds generally for active galactic nuclei, quantities derived from short samples will be subject to selection effects. Some naive interpretaions of variability are clearly invalid. Similar conclusions have recently been arrived at by McHardy *et al.* (see accompanying paper[6]).

NGC4051 was observed by EXOSAT for 62 h in December 1985, achieving a three-σ detection approximately every 50 s. Figure 1 shows the X-ray light curve, summed in 1,000-s bins. Variability is chaotic, and visually reminiscent of Cygnus X-1[7]. The flux ranges over a factor of 10, including a protracted episode of very low flux at the end of the observation. Figure 2 shows the power spectrum, calculated from the data summed in 50-s bins. At high frequencies ($>2\times10^{-3}$ Hz) the power spectrum is flat, variance being dominated by the Poisson noise of the data (indicated by a horizontal line in Fig. 2). At low frequencies (in the range 2×10^{-5} to 2×10^{-3} Hz) the spectrum is smooth and divergent at low frequencies, following a form close to $1/f$, where f is frequency. The shape is not necessarily exactly $1/f$, but for our purposes the important factors are the low-frequency divergence, and the fact that no preferred timescale stands out. We also cannot reject the possibility of modest curvature, but over the two decades which we can confidently measure, there is no substantial low-frequency flattening, or high-frequency steepening. The autocorrelation function (ACF) is consistent with our earlier measurement[2] at short lags, but does not confirm the 'zero-crossing time' of ~3,000 s, corresponding to the possible quasi-periodicity. The 3,000-s timescale is genuinely present in the earlier data, but our new data show that it is not characteristic of the underlying process. This discrepancy is typical of the problems that arise in short observations of low-frequency noise—structure always seems to appear on a timescale a few times shorter than the observation length.

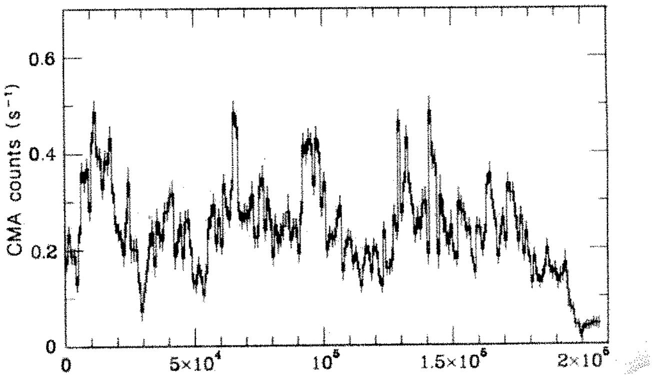

Fig. 1 X-ray light curve of NGC4051, commencing 1985 December 3 02:20 UT. Data presented here are from the EXOSAT[18] low-energy energy imaging system, the Channel Multiplier Array (CMA, ref. 19), using the thin Lexan filter, covering the energy range 0.05–2 keV. The source was also seen in the medium energy (ME, ref. 20) proportional counter array, showing variability closely similar to that seen here with the CMA. Bin size used is 1,000 s, error bars are $\pm\sigma$. Data were background-subtracted and exposure-corrected as in [2].

The result is spectacular, but also frustrating. Low-frequency divergent 'flicker noise' is common in a wide range of circumstances[8–11] and its cause is unknown. We may conclude that the

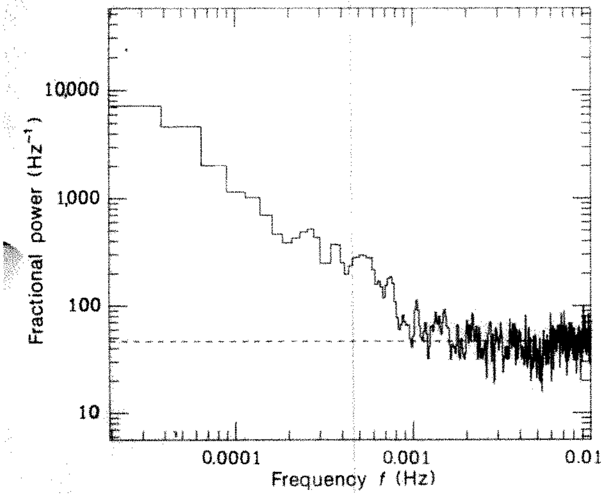

Fig. 2 Power spectral density, calculated by forming the autovariance function (ACV), applying a Hanning roll-off, and Fourier transforming. The ACV was calculated from data summed in 50-s bins (4,531 samples), and was truncated at a lag of 512 bins, so that the lowest frequency represented in the power spectrum is sampled ~5 times. The power per Hz has been divided by the total sample variance, calculated using the same bin size, to give fractional contribution per Hz to the total observed variance. This includes contributions due to both experimental error (that is, counting statistics) and to real source variability.

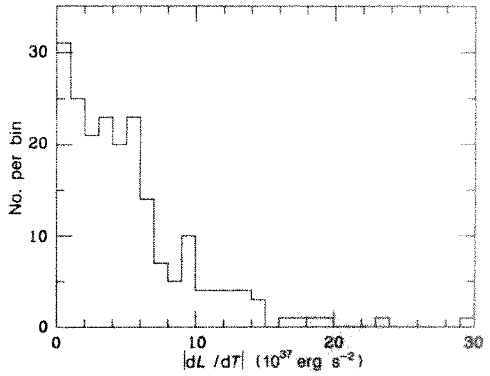

Fig. 3 Distribution of absolute values of dL/dt, in units of 10^{37} erg s^{-2}, calculated with 1,000-s bins.

X-ray emitting region is in a turbulent condition. There are also problems associated with the interpretation of variability, many of these arising from the pathological properties of $1/f$ noise[9]. We stress, however, that these do not depend on the spectral density exactly following $1/f$ over all frequencies, but only on its approximating $1/f$ in the observed regime. For a spectrum following $1/f^{\alpha}$, the power integrated to low frequencies diverges if $\alpha > 1$, and power integrated to high frequencies diverges if $\alpha < 1$. When $\alpha = 1.0$, power diverges at both ends. If the average logarithmic slope is close to 1, our sample estimates of total variance, mean and instantaneous values can be wrong by an unknown amount. Likewise, the local derivative of the time series diverges as one decreases the time step size, unless $\alpha > 2$.

Any timescale derived from such a light curve cannot be interpreted as a cooling timescale, synchrotron decay time, or similar, in order to constrain the physical properties of the emitting gas. All such effects give an exponentially decaying response to impulses, producing a power spectrum which is flat at low frequencies, and varies as $1/f^2$ at high frequencies[7]. (The logarithmic slope is equal to 1.0 at the knee frequency $f = 1/(2\pi\tau)$, where τ is the characteristic decay time, but the curvature is fast. A factor two above and below the knee frequency, the slope becomes 1.6 and 0.4, respectively, whereas the slope we see is close to 1 over two decades.) This means that models where variability is dominated by heating or cooling, for example, the Compton cooled hot cloud model for Cyg X-1[12], cannot explain $1/f$ variations.

Shot-noise models with exponential pulses can likewise be rejected. Shot noise can duplicate a $1/f$ spectrum if the shots are of a suitable shape (decaying as the square-root of time), or if the shots are exponential but have a range of decay times, with distribution function $D(\tau) \sim 1/\tau$. Similarly, heating-cooling models can be rescued by postulating a range of timescales. However such formal solutions are arbitrary unless the formalism is derived from a physical model.

There is no preferred timescale. The frequently used 'doubling timescale' is not well defined. For example, at the beginning of the observation, there is a decline by a factor of four over ~20,000 s, but towards the end of this, a sharp rise by a factor of two in ~1,000 s. In an observation of worse signal-to-noise, only the slow decay would be seen. The 'fastest doubling timescale' as used by Barr and Mushotzky[5] thus depends on data quality and coverage. (Given an instrumental time resolution limit, one only has to wait long enough before seeing a factor of two change.) Furthermore, only for exponential events will the doubling timescale deduced from large and small amplitude changes be identical.

The shape of the power spectrum also implies problems for another quantity of interest, dL/dt, the rate of change of luminosity, which can be used in model-independent formulae to derive the efficiency of energy generation, and the compactness parameter[13]. The arguments used are weak if applied to individual fluctuations, so one should rather look for a characteristic value of $|dL/dt|$. Unfortunately, within the $1/f$ regime, the derivative diverges towards smaller timescales. So here again, the result found for dL/dt depends on data quality. (We confirmed this numerically, by making dL/dt light curves for various time resolutions, and comparing the dL/dt distributions.) Even for a given time resolution, it is not clear what quantity should be considered a 'characteristic' amplitude for dL/dt. The distribution of values of dL/dt is symmetric about zero, indicating no preference for rises to be faster than decays. The distribution of $|dL/dt|$ has a low modal value, but with a long tail of fluctuations many times larger than the mode. Figure 3 shows the distribution of $|dL/dt|$, using the data in 1,000-s bins. It is possible to derive a lower limit, but the problem of what to take as typical remains. At a resolution of 50 s, the largest values of dL/dt that we see are of the order of 10^{40} erg s^{-2} (luminosity expressed in the range 0.1–1.0 keV, assuming spectrum as in ref. 2). A related quantity often used to define an 'objective' timescale is $L/(dL/dt)$. For a given time resolution, this does define a local timescale, but tends to zero at small times, and has no obvious characteristic value for the process as a whole. Overall, that we have such problems defining an objective timescale does not mean that no meaningful quantities with the dimensions of time can be derived from such data, but that we need to specify a model of the temporal process first (preferably deriving from a physical model of the object).

The power spectrum measures the variance (and thus the typical size of variability) at a given timescale, so we can in principle compare active galactic nuclei (AGN) with each other, or with objects like Cyg X-1. However, the absolute size of variance depends on the apparent flux of the source, so it is necessary to normalize the power spectrum to the total variance, or equivalently to normalize the light curve to the mean flux. As explained above however, we cannot be confident that we have yet made fair estimates of the variance or the mean, and thus of the normalized power spectrum. It may be that the process producing $1/f$ variations is not statistically stationary. If however we assume stationarity, our problems will be alleviated if the spectrum eventually flattens at low frequencies and steepens at high frequencies. A flattening at low frequencies is seen in Cyg X-1 and GX339-4[10,17]. The position of such a knee would define an unambiguous timescale, to compare with Cyg X-1 or GX339-4, but of course, as in these latter cases, such a timescale would be very long, and of no relevance to estimating black hole masses, or Doppler boosting factors. (It may tell us something about the gross geometry and/or physical conditions of the accreting regions.) We do have a limit on the knee position, $<2\times10^{-5}$ Hz ($n=-5$). In Cyg X-1 the knee is at $\sim5\times10^{-2}$ Hz (ref. 7). If knee position scales with X-ray luminosity, the expected position is $\sim5\times10^{-6}$ Hz, so we are just short of checking this hypothesis. Even more frustratingly, it seems to be normal when publishing astronomical power spectra to label the ordinate 'power in arbitrary units', for example with Cyg X-1[7] and GX339-4[14]. So, even if we ignore the divergence of variance and use the sample variance to normalize the spectrum, we cannot currently tell if the power spectrum of NGC4051 is consistent with the power spectrum of Cyg X-1, with frequency scaled by the ratio of luminosities.

There have been two optical studies of AGN with which the present results can usefully be compared. First, Moore *et al.*[15] have monitored the *V*-magnitude and polarization of BL Lac over a period of one week. The spectrum is low-frequency divergent, but a more detailed comparison is hard. No units are given for the power spectrum; the slope seems closer to $1/f^2$ than $1/f$, but the scatter is large; and the dynamic range over which there is statistical confidence is only a factor of 10. The second AGN comparison worth making is with the historical optical light curve of 3C273. Kunkel[16] presents a power spectrum which again seems close to $1/f^2$ (the $1/f^2$ form jusified Terrell and Olsen[17] in fitting a shot-noise model). Kunkel's figure also shows an apparent low-frequency flattening, but this is probably not real. As the total sample length is 80 yr, the sample power spectrum will be a poor estimator (and probably biased) below ~0.05 cycles per year.

We have emphasized that to extract physical parameters from an erratic time series, it is necessary to propose a model which specifies an expected temporal form. Certainly most earlier simple interpretations of variability, and its systematics, need to be treated with great caution. From this point of view, the failure of physicists to produce models which convincingly explain $1/f$ noises in other circumstances[10,11] is depressing. However, we can reject models (for example, simple shot noise models, models dominated by a single cooling or heating timescale), and the fundamental limits remain (for example, light travel time). Also, the fact that AGN flicker noises at various wavelengths and timescales are not all $1/f$ raises some hope that systematic differences may yield clues. Finally, we have the hope that extension to longer and/or shorter timescales will finally reveal characteristic features.

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Fractal X-ray time variability and spectral invariance of the Seyfert galaxy NGC5506

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Rapid X-ray variability has long been sought after in active galactic nuclei (AGN) as an indicator of such parameters as the mass-to-energy conversion efficiency and black hole mass. Although some interesting variability information has been gained from early observations (for example see ref. 1 for a review) the faintness of AGN, combined with the short times devoted to their observation, has resulted in a very sparsely sampled and consequently partially misunderstood picture of AGN variability. The launch of EXOSAT[2] in May 1983 provided us with the first opportunity to study seriously AGN X-ray variability and significant variations have been detected in a number of short observations[3,4]. Here we report the results of a long (3-day) observation of NGC5506 in which continuous rapid variation was detected. Power spectral analysis and fractal analysis both demonstrate that the variability is self-similar, that is, scale invariant, over nearly three decades of frequency, a range similar to that covered by pre-EXOSAT observations of bright galactic sources. If this behaviour is typical of all AGN then the concept of a 'two-folding' timescale is meaningless unless the fractal dimension of the general variability, as well as the specific time interval over which a specific change in flux occurs, are all noted. There is no evidence for any variation in the X-ray spectrum as a function of intensity and we show how this places strong constraints on the emission mechanism, favouring non-thermal models involving shocks.

The moderate X-ray luminosity ($L_x[2\text{–}10\,\text{keV}]=1.4\times10^{43}$ ergs s^{-1}) Seyfert 2 galaxy NGC5506 was observed continuously by EXOSAT for 3 days from 24–27 January 1986 and was detected strongly in the medium energy (ME) detectors[5] but only weakly through the thin lexan filter of the low-energy imaging telescope (LE)[6]. The highest observed signal-to-noise region of the X-ray spectrum of NGC5506 is that between 2 and 7 keV in the ME detectors, and so we present the background-subtracted light-curve in this energy range in Fig. 1. Variability on all timescales is apparent. The power spectrum of the light-curve is presented in Fig. 2. The power spectrum has a slope of ~-1 (that is, $1/f$) in the frequency range $10^{-4.8}$–$10^{-3.5}$ Hz, similar to that of the bright galactic X-ray source Cygnus X−1 (ref. 7). At higher frequencies the slope is harder to define accurately, but may steepen briefly before flattening off as it becomes dominated by Poisson noise at frequencies above $\sim10^{-3}$ Hz. There are no obvious strong features in the power spectrum, such as the characteristic flattening in the power spectrum of Cygnus X−1 (ref. 7) at 5×10^{-2} Hz. However the fact that the power spectrum is described reasonably by a power-law does tell us that there are no preferred timescales and that variability occurs in a scale-invariant manner, at least over the range $10^{-4.8}$–$10^{-3.5}$ Hz.

We now introduce another characteristic of the variability by calculating the fractal dimension of the lightcurve. (See Mandelbrot[8] for a general introduction to fractals.). This parameterization was prompted by the similarity of the X-ray light-curve to the shape of a coastline, whose degree of irregularity is well described by a fractal dimension. A full discussion of the applicability of fractal analysis to the understanding of variability is postponed to a future paper and here we merely quote our definitions of fractal 'length' and fractal 'dimension'.

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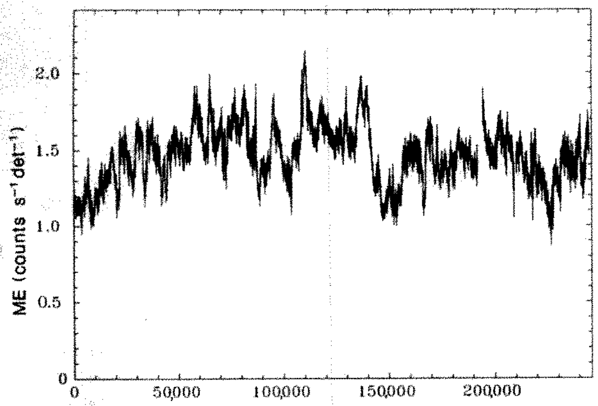

Fig. 1 X-ray light-curve of NGC5505 obtained with the EXOSAT ME in the energy range 2–7 keV. Bin size is 400 s. Error bars of $\pm 1\sigma$ are shown. $T=0$ corresponds to 1986 January 24 21:43:32 UT.

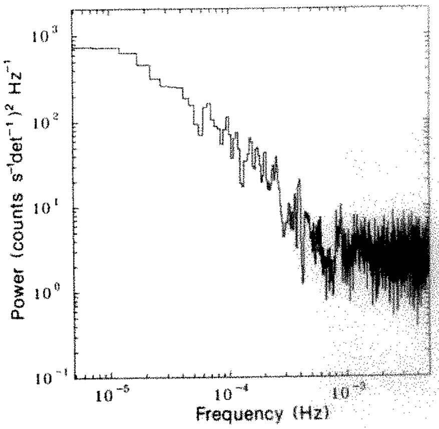

Fig. 2 Power spectrum of the light-curve shown in Fig. 1.

We define a length $L(\Delta t)$, as a function of measuring timescale, Δt, by

$$L(\Delta t)=\frac{1}{\Delta t}\int_0^T |F(t+\Delta t)-F(t)|\,\mathrm{d}t$$

and hence define a fractal dimension, D, for the light-curve by

$$D=\frac{-\mathrm{d}\log L(\Delta t)}{\mathrm{d}\log \Delta t}$$

Here, our dimension varies between 0 and 1 rather than between 1 and 2, as does that of a coastline, because we are considering only a (Euclidean) one-dimensional problem, that of the variation of intensity.

The measured value of $\log L(\Delta t)$ contains a contribution from the noise associated with the flux measurements. This can be calculated very simply (it has $D=1$), given the known value of the errors. The noise is statistically independent of the signal and so can be subtracted using the relation

$$L_{\text{observed}}=(L^2_{\text{Signal}}+L^2_{\text{Noise}})^{1/2}$$

We performed the subtraction for three possible values of the noise associated with each measurement: (1) the average value of the error associated with each flux measurement, 0.109 counts s^{-1} det^{-1} (filled circles); (2) zero noise subtracted (open squares); and (3) an average noise value of 0.131 counts s^{-1} det^{-1} (stars). This latter value is chosen somewhat arbitrarily but corresponds to the largest value that can be subtracted without making $L_S(100\,\mathrm{s})<0$. The resultant plot of $\log L_S(\Delta t)$ against $\log(\Delta t)$, after subtraction of the noise, is shown in Fig. 3.

Log L_S appears to be linearly related to $\log \Delta t$ down to $\Delta t=100$ s, although for $\Delta t \leq 400$ s the envelope provided by the allowed error in our determination of the noise cautions against placing too much confidence in the relationship. However for $\Delta t > 400$ s the relationship is well constrained and remains so up to the limits of observation (10^5 s).

We have shown that the X-ray variability of NGC5506 is well described by a fractal dimension (0.6) over nearly 3 decades in frequency and therefore that the variability is self-similar, and hence scale-invariant, over this range. The fractal dimension we measure is close to that of (Euclidean one-dimensional) turbulence (0.5) and so similar random processes, such as randomly separated outbursts of random amplitude, are probably responsible for the irregular shape of the X-ray light-curve. The same result is broadly understandable from consideration of the large amplitudes visible in the light-curve; for these to be produced by random superposition of many of the smaller amplitudes we see is statistically extremely unlikely.

The fractal dimension cannot remain greater than 0.5 down to infinitesimally small timescales as this would imply infinite energy. However the present data tell us that any decrease in the dimension, that is, anything which we might associate with a minimum timescale, must occur at timescales shorter than 400 s. Thus, until we can measure deviations from self-similarity, we can only place a lower limit on the rate of change of luminosity with time. The limit in this case ($\sim 10^{39.5}$ ergs s^{-2}) only implies an efficiency of mass-to-energy conversion of 0.5% and hence places no strong constraints on black hole models.

It can be shown (D. Raine, personal communication) that, within certain limits, if a power spectrum has a power-law slope, P, the corresponding fractal plot will have a dimension, D, where

$$P=2(D-1).$$

Thus, the fractal dimension we measure here agrees, within the uncertainties, with the value $P=-1$, that is, $1/f$ noise, which we directly measure from the power spectrum (Fig. 2) over the range $10^{4.8}$–$10^{3.5}$ s.

The scale-invariant temporal variability of NGC5506 makes it impossible to identify any particular timescale as, for example, a dynamical or thermal timescale. If this is a problem common to active galaxies in general it will not be possible to calculate such parameters as a black hole mass from the characteristics of the variability. Another frequently discussed parameter, the two-folding timescale, also becomes meaningless for self-similar light-curves, as can easily be seen from fractal analysis. For $D\neq 0$ the local derivative is not determined and the value which we might try to define as the derivative, $\Delta F/\Delta t$, depends on Δt with a mean value of

$$\left|\frac{\Delta F}{\Delta t}\right| \propto \Delta t^{-D}$$

independent of the actual value of F. However for self-similar variability F can, in principle, take any value. Thus the mean value of F we measure during any observation will depend on when the observation occurred and so any timescale defined by $F/(\mathrm{d}F/\mathrm{d}t)$, as is conventionally the case, will also depend on the local mean value of F. Only if we restrict ourselves to considering changes within a well-observed time interval, or if self-similarity breaks down at large times and the mean value of F is approximately constant, can we define a universal two-folding time for a source. In this case we still need to measure a change by a factor of 2 to define such a timescale. If the observed change was smaller or larger than a factor of 2, we would have to determine the dimension of the variability and apply a correction factor reflecting the dependence of $|\Delta F/\Delta t|$ on Δt. For example a two-folding timescale, Δt_2, can be calculated from observed variations in flux of 10% in a time Δt_1 by

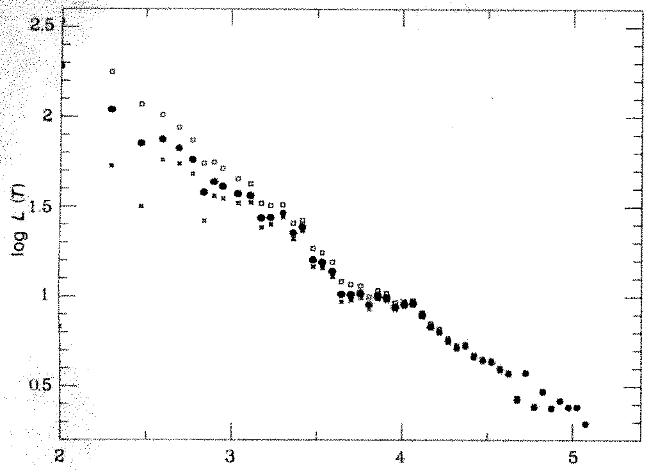

Fig. 3 Shows log of total 'length' plotted against 'timescale' (as described in the text) for the light-curve shown in Fig. 1. The slope of the curve is $-D$, where D is the fractal dimension.

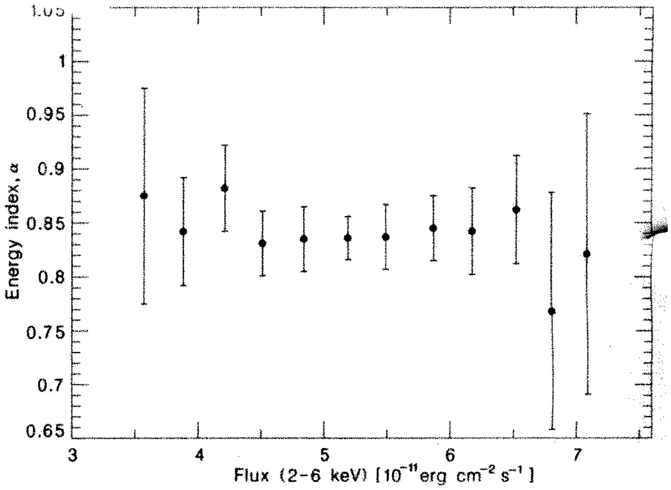

Fig. 4 Power-law spectral index as a function of intensity.

$\Delta t_2 = \Delta t_1/(0.1)^{1/(1-D)}$. The relationship between two-folding time and luminosity shown by Barr and Muchotzky[9] needs to be redrawn with this correction applied.

The source is heavily cutoff ($N_H = 2.8 \times 10^{22}$ cm^{-2}) and the average LE count rate (1.9×10^{-3} counts s^{-1} [0.05–1.0 keV]) is in very good agreement with a simple extrapolation of the ME spectrum to low energies. The LE count rate is too low for us to study low-energy variability; however, the ME count rate is high enough to allow us to investigate the variation of the medium-energy spectrum as a function of intensity. We have therefore divided the observation into twelve equally separated intensity levels covering the full range of variability and have fitted simple power-law plus absorbing column models to the data in each level. The resultant spectra revealed no evidence for change in either the absorbing column or energy index. If we assume that the absorbing column does not change, which seems reasonable as the timescale for large-intensity variations is very short (~hours), we can fix the absorbing column at its best-fit value [2.8×10^{22} cm^{-2}] and so derive improved estimates of the variation in power-law index. In Fig. 4 we plot the variation of index thus derived (here, for computational ease, we show only those data derived from half 2 of the ME). There is no evidence at all for any variation. The best-fit value is 0.84 ± 0.04. The spectral parameters we derive are in reasonable agreement with previous determinations[10,11] and indicate that there are no long-term spectral variations in this source.

We have constructed a hardness ratio (not shown) between the counts in the energy range 4.5–7 keV and 2–4.5 keV. The ratio is approximately constant throughout the observation and certainly does not follow the rapid rises and falls apparent in Fig. 1. Therefore the constancy of the spectral index shown in Fig. 4 is not a result of a superposition of, say, a harder spectrum during outbursts and a softer spectrum during declines; rather, the spectrum must be approximately constant at all times.

By means of fractal and power spectral analysis we have shown that the X-ray variability of NGC5506 is self-similar, or scale invariant, over nearly 3 decades of frequency with a fractal dimension close to that of random processes such as turbulence. Such random activity could be accounted for in many ways; for example by the release of stored energy in magnetic fields or perhaps pairs, by turbulent accretion and associated Doppler enhancements or by shocks. Any such activity must not, however, produce any variation in the observed X-ray spectrum and so 'constant seed' (that is, variable temperature) thermal Compton models[12] can be rejected.

Simultaneous optical photometry was obtained for part of the X-ray observation by Ricker and Vanderseck. These data are not yet fully reduced but initial indications are that no variability amplitudes as large as those shown in Fig. 1 were detected. If full analysis confirms this, 'constant temperature' (and hence variable seed) thermal Compton models may also be in some difficulty (see NGC4051 where Lawrence *et al.*[3] indicate that seeds may come from the optical region).

The release of stored magnetic field energy into the thermal energy of a plasma, such as occurs in RS CVn systems[13], can also probably be rejected as this leads to a rise in temperature during the outburst and a fall during the decline. The fact that the X-ray spectrum fits a power-law very well (but is not well-fitted by a simple thermal bremmstrahlung model) also argues against release of stored magnetic energy, as does the fact that the exponential decay of such bursts would produce a power spectrum which is not a simple $1/f$ power-law[2].

If the source is a regime where pairs are important, a slight change in the gamma-ray luminosity, such as may accompany a variation in accretion rate, can be reflected in a large variation in the X-ray luminosity with little or no change of spectral slope[14].

Finally we note that perhaps the simplest method of producing spectrally invariant intensity variations is with shocks. If the underlying electron distribution has a power-law form then simple compression and expansion of the radiating regions will alter the intensity (of synchrotron or synchrotron self-Compton emission) without change of slope. Even if the initial electron distribution is not a power-law, then shocks are likely to turn it into one eventually.

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Scintillation of a radio source observed through the tail of comet Halley

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On 29 March 1986 between 16:00 and 23:00 UT we observed the strong compact radio source 1827–360 as the plasma tail of comet Halley swept across the source. We noted the appearance of weak intensity fluctuations during the interval 17:00 to 19:00 UT when the Sun, the comet's nucleus and the radio source were accurately aligned. We interpret this behaviour as the result of diffraction of radio waves by electron-density irregularities in the plasma tail. At a distance 5.4×10^6 km downstream from the cometary nucleus the central part of the plasma tail contained electron density irregularities about 14 times as strong as those in the undisturbed solar wind.

The source 1827 – 360 is known to exhibit interplanetary scintillation[1] and has the turnover in the low-frequency radio spectrum[2] often shown by very compact radio sources. The high scintillation flux[1] of 10.6 Jy out of a total 408 MHz flux density[3] of 25.8 Jy makes it an ideal source for detecting scintillation from the plasma tail of Comet Halley. The observations were made with a dual-channel 408 MHz receiver installed on the Parkes 64-m telescope. The system temperature of each channel was ~120 K and bandwidth 5 MHz. The operation of the telescope and data taking were computer-controlled; the total power in each polarization channel was sampled at 50 Hz in blocks of 40.96 s and the readings stored for subsequent analysis. Active low-pass filters with a cut-off at 23 Hz minimized aliasing of frequencies above the Nyquist frequency of 25 Hz. Off-line analysis consisted of: (1) subtracting a quadratic baseline from each block of data and rejecting interference spikes; (2) plotting the modified data and computing its r.m.s. variation; (3) computing a power spectrum with frequency resolution of ~0.05 Hz; (4) averaging the two polarization channels and averaging several data blocks.

The path of the comet projected on to the plane of the sky is shown in Fig. 1. The nucleus of Halley had a geocentric velocity of 39.94 km s^{-1} and its elongation from the Sun was 89°. The topocentric and heliocentric distances were 0.576 and 1.143 AU respectively; the projected distance from the nucleus at which the tail passed in front of the source was 5.4×10^6 km.

Figure 2*a* shows a block of data taken at 17:35 UT with the telescope pointing 1° away from 1827 – 360; Fig. 2*b* shows a block of data taken at 18:10 UT while pointing at the source. The noticeably increased noise fluctuation level in Fig. 2*b* yields a scintillation index (defined as m = r.m.s. variation in flux/scintillating flux density) of $m = 0.064$. Figure 3 is a plot of the scintillation indices measured over the four observing days. The scintillation index has been computed by subtracting the variance off source from that on source, with a correction to account for the 12.5% increase in the system temperature when on source. Each scintillation index is an average over 15 min, the larger gaps on 29 March being due to off-source checks.

The scintillation index on 29 March was significantly higher for much of the 7.5-h observing interval than it was on the preceding and following days. In particular, during the interval 17:00–19:00, when Fig. 1 shows that the centre of Halley's plasma tail was passing in front of the source, the scintillation index reached 0.062, which is about four times higher than the average of 0.016 for the other three days. Other features of Fig. 3 are the very low scintillation indices between 19:30 and 21:00 UT and a secondary maximum of ~0.040 between 22:00 and 22:45 UT.

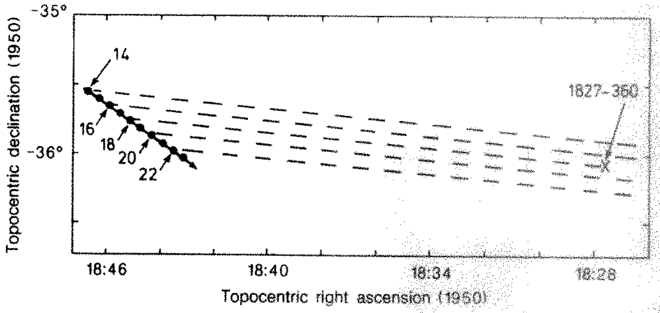

Fig. 1 Equiangular projection on to the plane of the sky of the path of Comet Halley with respect to the radio source 1827 – 360 (cross). The position of the nucleus is shown at hourly intervals (UT) on 29 March 1986. The dashed lines represent the extended Sun-Halley vectors at selected times.

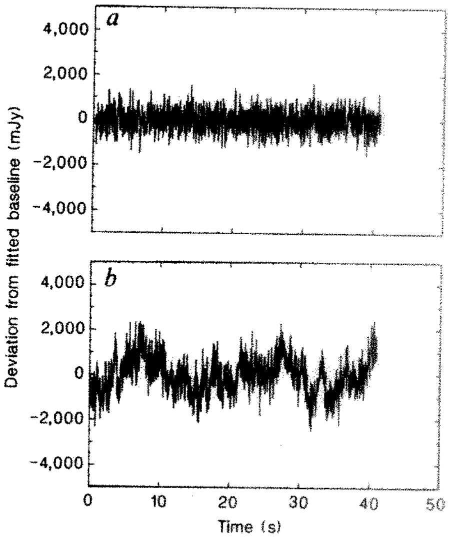

Fig. 2 Samples of data taken during the occultation. Panel *a* refers to a block of data recorded off source at 17:35 UT on 29 March 1986; *b* shows a block of data recorded on source at 18:10 UT.

We have examined the possibility that ionospheric scintillation may have been responsible for the peaks in Fig. 3 by checking the F2 critical frequencies and frequency spreading determined by a sounder operated by the Australian Ionospheric Prediction Service at Canberra, ~300 km SSW of Parkes; the radiation from 1827 – 360 would have been passing through the F2 layer above Canberra near the time of the maximum in scintillation index from 17:00 to 19:00 UT. The 15-min ionograms showed a reasonable degree of spread-*f* throughout our observing interval, but the spreading was most severe between 18:30 and 19:45 UT when, in fact, the measured scintillation index was dropping rapidly. The geomagnetic K_p indices of 3, 2+ over the observing interval on March 29 were close to those on neighbouring days. These considerations, together with the fact that the peak in the scintillating power spectrum (see Fig. 4) occurs at ~0.1 Hz (much too high for normal ionospheric scintillation), effectively eliminates an ionospheric origin for our scintillations.

We do not believe that our results can be explained by the chance occurrence of an enhanced patch of solar wind turbulence, because: (i) the plasma density and velocity in the solar wind as measured by the spacecraft Sagigake[4] were particularly stable, with values characteristic of the undisturbed plasma for many days in late March and early April 1986; (ii) comparison observations of 1827 – 360 on three days (see Fig. 2) gave very low values of scintillation index; (iii) the scintillations were strongest when the extended Sun–comet vector was passing over the source.

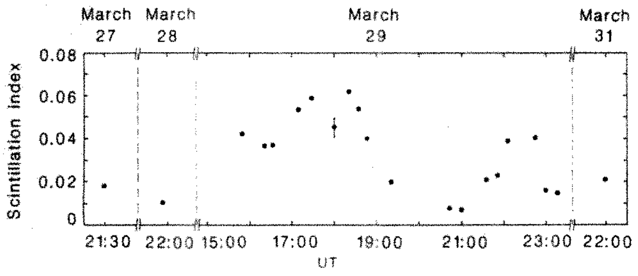

Fig. 3 Scintillation indices computed from data taken over the four days on which 1827 – 360 was observed. Each point represents an average over ~15 min. A typical standard error bar is shown. Off-source measurements were made during the longer gaps on 29 March.

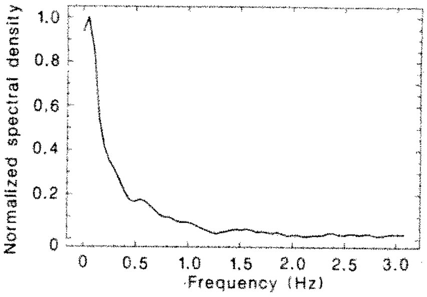

Fig. 4 A scintillation power spectrum computed from ~1 h of on-source data near the peak of scintillation index in Fig. 3. The frequency resolution before Hanning was 0.0488 Hz. Spectral estimates have been normalized to the maximum value.

Figure 4 shows the scintillation power spectrum obtained by averaging over ~1 h of data near the peak of scintillation index in Fig. 2. The spectrum was computed by subtracting the average of 60 off-source spectra from the averge of 80 on-source spectra. The spectral resolution before Hanning is 0.0488 Hz and the spectral densities have been normalized to the maximum value. It is clear that most of the variance is contained in frequencies below 2 Hz. This spectral behaviour is similar to that of ordinary interplanetary scintillation in the weak scattering regime[5] except that more of the variance is at lower frequencies. We have made computations of scale size and r.m.s. electron density fluctuation in the comet tail on the very reasonable assumption that we are dealing with weak scattering in a thin diffracting screen.

We have no photograph of Halley's plasma tail on the night of our observation (near full moon), but a UK Schmidt plate taken by D. Malin (personal communication) on 12 March gives a good impression of the structure that was probably present. At the distance of 5.4×10^6 km from the nucleus relevant to our present analysis the bright central part of the plasma tail had a projected width of 4.8×10^5 km and would have drifted across the radio source in ~3 h. From the scattering theory developed by Salpeter[6] the correlation length or scale size is given by $a = v/2\pi f_2$, where v is the velocity of the diffraction pattern across the observer and f_2 is the width of the scintillation power spectrum at the $\exp(-0.5)$ point. It is unlikely at a distance 5.4×10^6 km (0.036 AU) downstream from the nucleus that the cometary plasma would have been accelerated to the solar wind velocity. Observations of plasma condensations in the tail of comet Kohoutek by Jockers[7] yielded velocities <100 km s^{-1}. Here we assume that the total translational velocity of the plasma with respect to the line of sight to 1827 – 360 was $v = 90$ km s^{-1}, which, when combined with $f_2 = 0.14$ Hz measured from the power spectrum of Fig. 4, gives $a \approx 100$ km. This scale size is typical of ordinary interplanetary scintillations when radio sources are observed in the weak scattering regime[5]. For a gaussian electron density correlation function the r.m.s. phase deviation across the wavefront emerging from the screen[5] is

$$\phi_0 = (2\pi)^{1/4} r_e \lambda (aL)^{1/2} \Delta N,$$

where $r_e = 2.82 \times 10^{-13}$ cm is the classical electron radius, L is the thickness of the screen and ΔN is the r.m.s. electron density deviation. For weak scattering the scintillation index[6] is $m = \sqrt{2}\phi_0$, from which in our case ($\bar{m} = 0.062$) $\phi_0 = 0.044$ rad. Then making the reasonable assumption that the screen thickness is approximately equal to the width seen on the photograph of 12 March ($L \approx 4.8 \times 10^5$ km) we deduce that $\Delta N \approx 1.9$ cm^{-3}. This value is an order of magnitude higher than the value of ΔN ordinarily found in the solar wind at heliocentric distances of ~1 AU (see, for example, Fig. 9 of Cohen *et al.*[5]). We have also estimated ΔN by using the more realistic Kolmogorov power-law spectrum of irregularity sizes. Equations (16) and (17) given by Lee[8] with an inner scale of 100 km (the scale size from the gaussian analysis) and an outer scale of 4.8×10^5 km (the width of the plasma tail) were solved to give $\Delta N = 1.8$ cm^{-3}, which is unexpectedly close to the value from the gaussian spectrum.

Scintillation experiments do not provide a direct measurement of the averge electron density in a comet tail. If the r.m.s. electron density deviation which is responsible for the scintillations is proportional to the average electron density, then at 5.4×10^6 km downstream from the nucleus on 29 March the average electron density was ~70 cm^{-3} (14 times the solar wind value of ~5 cm^{-3} measured[9] under similar undisturbed conditions upstream from Halley during Giotto's approach on 14 March 1986.

It is instructive to compare our results with those of Ananthakrishnan *et al.*[10] from comet Kohoutek and Alurkar *et al.*[11] from the recent apparition of comet Halley. Lee[8] has shown that the 327 MHz scintillations observed during the occultation of PKS2025 – 15 by the tail of Kohoutek at a distance of $\sim 5.5 \times 10^5$ km (0.00 37 AU) from the nucleus could be explained by a Gaussian spectrum of turbulence having a scale of $a \approx 800$ km and $\Delta N \approx 22$ cm^{-3}. These values should be compared with those from our experiment of $a \approx 100$ km and $\Delta N \approx 1.9$ cm^{-3} at a distance of 5.4×10^6 km (0.036 AU) from Halley's nucleus.

A better comparison is with the 103 MHz scintillations[11] of PKS2314+03 during occultation by Halley's tail in December 1985. Postulating a gaussian irregularity spectrum with $a \approx 100$ km, these authors claim a rapid variation in ΔN with radial distance along the tail from $\Delta N = 10$ cm^{-3} at 0.12 AU to $\Delta N = 3$ cm^{-3} at 0.18 AU. In subsequent correspondence S. K. Alurkar *et al.* (personal communication) have agreed that their assumed tail width of 10^5 km is probably underestimated by a factor of approximately five, so that ΔN should be reduced by $\sim\sqrt{5}$. The evidence that this is a real radial variation in ΔN is not very convincing—the observations occupied only ~10 min each day and we know from Jockers'[7] observations of comet Kohoutek that plasma condensations move out along the tail with sufficient velocity to change their radial distribution from day to day. We prefer to average the 103 MHz results to give $\Delta N \approx 2.8$ cm^{-3} at 0.15 AU from the nucleus. Therefore, assuming that rate of ionization of nuclear material had not changed significantly from December 1985 to March 1986, there is little, if any, convincing radial variation in ΔN.

The results from comet Kohoutek[8,10] are not compatible with the Halley measurements. The 103 and 408 MHz measurements of Halley indicate that if there is a radial gradient in ΔN, it is in the direction of the turbulence level decreasing slowly with approach to the nucleus. On the other hand, the r.m.s. electron density for comet Kohoutek at 0.0037 AU from the nucleus is an order of magnitude higher than an extrapolation of our results inward would suggest.

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Protracted climatic effects of massive smoke injection into the atmosphere

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Climate perturbations caused by sudden injections of aerosols into the atmosphere have received increased attention with the realization that fires started by a nuclear war might release sufficient quantities of smoke to block sunlight from the Earth's surface and dramatically reduce temperatures over land[1–3]. Three-dimensional atmospheric general circulation models which include aerosol transport indicate that much of the smoke could survive in the atmosphere for months or more[4–6], implying significant climatic effects on such timescales, but these models generally assume fixed sea surface temperature and sea ice, and therefore cannot properly simulate cooling for more than a few weeks. This report describes a general circulation model run in which the foregoing assumptions are relaxed and the climate impact of massive smoke loading is assessed for four months of simulated time. Two competing long-term effects are apparent: positive feedback on surface cooling due to enhanced sea ice formation, and surface warming at some latitudes due in part to heat transport from the upper troposphere, where smoke aerosols absorb sunlight.

It is instructive to compare results from the general circulation model (GCM) to those obtained by Robock[7] using a one-dimensional (in latitude) energy balance model (EBM) in multi-year simulations of nuclear war climate effects. In the present study, total column absorption optical depth of smoke τ is prescribed as a function of time to be twice the value normally assumed by Robock except that we restrict $\tau \leq 3$, that is, underneath the smoke $\tau = 3$ for the first 30 days and thereafter τ decreases with a half life of 30 days. This prescription ensures that GCM results of interest are obviously significant compared to model-generated variability (that is, weather fluctuations) and also that during the first 20 days after aerosol injection the smoke concentrations assumed for this study are identical to those used in our earlier GCM experiments with prescribed sea surface temperature (SST)[8,9]. In all studies discussed here, including Robock's, smoke is assumed to exist in a uniform band of latitudes between 30° and 70° N and is injected into the atmosphere at approximately the Northern Hemisphere spring equinox. In the GCM runs smoke is assumed to be distributed between 1 and 10 km altitude at constant mixing ratio.

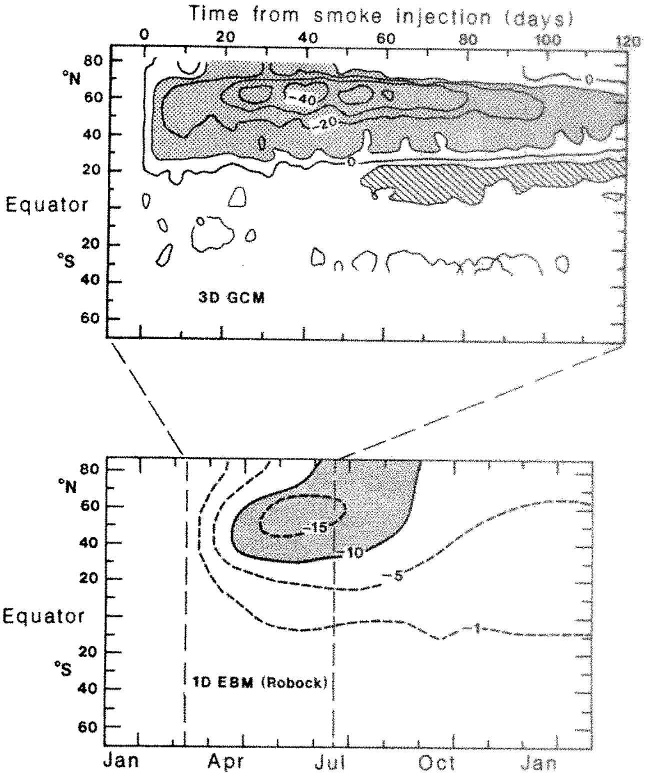

Fig. 1 Zonally averaged land surface temperature difference in °C between prescribed smoke experiments and controls. The upper panel shows results obtained in the present study; the lower panel is redrawn from ref. 7 and shows results from a one-dimensional energy balance climate model. In both cases solid line contours are drawn at 10 °C intervals, and shaded areas indicate temperatures more than 10 °C cooler than control runs while hatched areas indicate temperatures more than 10 °C warmer than control runs. GCM results are shown for 130 days and the thin dashed lines indicate the corresponding time period in Robock's simulation. Results from the GCM are not plotted at latitudes which are more than 90% ocean.

The assumed initial condition $\tau = 3$ in the Northern Hemisphere mid-latitudes is equivalent to 34×10^{12} g of amorphous elemental carbon with absorption cross section $10\ \mathrm{m^2\ g^{-1}}$ and corresponds to 'baseline' estimates of nuclear war smoke production published by the US National Academy of Sciences[2] and the International Council of Scientific Unions[3]. However, longer term optical depths, $\tau = 2.4$ at 40 days and 0.5 at 110 days, greatly exceed values implied by GCMs which compute smoke removal[5–6,10], and or course the assumption that smoke is fixed in space is unrealistic. Thus, the present study is an intercomparison of climate model responses to hypothetical perturbations, with application to nuclear war climate effects; it is not an attempt to directly simulate 'nuclear winter'. The use of extreme smoke amounts and the assumption that smoke does not spread beyond the Northern Hemisphere mid-latitudes mean that long-term temperature effects are probably overestimated by the present study. Indeed, in reporting a long-term 'nuclear winter' simulation including smoke spreading by a two-level GCM, Stenchikov[11] does not elucidate the surface temperature feedback processes discussed below, perhaps because these processes are less obvious with more realistic (smaller) smoke concentrations.

The GCM used in this study is the NCAR (National Center for Atmospheric Research) Community Climate Model (CCM)[12]. Results presented here were obtained with a research version of the CCM developed by Washington and Meehl[13,14] for studies of the CO_2 greenhouse effect. In this model SSTs are computed assuming a static mixed layer of constant heat capacity, and sea ice is computed with a simple thermodynamic model. This GCM, hereafter referred to as the CCM/mixed layer model, was modified as described by Covey *et al.*[8,9] to include absorption of sunlight by smoke. Similar representations of mixed layer heat capacity and sea ice are used in Robock's EBM. (The earlier studies of Covey *et al.*[8,9] made use of a version of the CCM in which SST and sea ice are prescribed at seasonally varying climatological values[15]; this model will be referred to as the prescribed SST version.)

Figure 1 shows the smoke-induced land surface cooling (that is, the temperature difference in °C between prescribed smoke experiments and controls) averaged over longitude and plotted as a function of latitude and time. The upper panel shows results

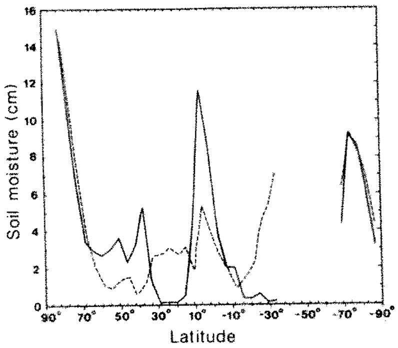

Fig. 2 Soil moisture content averaged over longitude and over days 110 to 120 after smoke injection (that is, the final 10 days of the GCM simulation) for the smoke-perturbed case (solid line) and the control case (dashed line). Results are not plotted at latitudes which are more than 90% ocean.

from the CCM/mixed layer model and the lower panel shows results from Robock's EBM ('Run 4' of ref. 7). During the first half of the 120 day simulation with the CCM/mixed layer model, land surface cooling underneath the assumed smoke cloud is about twice that exhibited by Robock's model. Maximum cooling in the GCM is 47 °C at day 37 and 60° N latitude (poleward of the sea ice margin, slightly to the north of maximum cooling in Robock's model). During this time period smoke optical depths assumed in both the CCM/mixed layer model and Robock's simulation are >1, so the discrepancy in the amount of cooling between the two models is difficult to explain by the difference in smoke loading. The CCM/mixed layer model may exaggerate the amount of cooling because it overpredicts the unperturbed amount of sea ice, possibly due to neglect of poleward heat transport by the oceans[13,14]; the maximum temperature drop exhibited by the CCM/mixed layer model is almost twice that of the prescribed SST version during the time that smoke loadings in the two GCMs are identical. On the other hand, Robock's model may well underpredict the maximum degree of land surface cooling because its limited resolution— 10° latitude, no longitude resolutions within land and ocean sectors, no height resolution—requires the cooling to be spread out over a large volume of space. The latter consideration may also explain in part the rapid cooling exhibited by the CCM during the first few days after aerosol injection[8]; the EBM, in contrast, takes several weeks to obtain land surface cooling >10 °C. Another factor contributing to rapid initial cooling in the GCM is atmospheric dynamics, not included in the EBM. An examination of the lower tropospheric wind and temperature fields (not shown) indicates that ocean-to-land temperature advection takes about two weeks to increase to its maximum value as the circulation in the perturbed case evolves to a more zonally symmetric state. Two weeks is approximately the thermal relaxation time of the lower troposphere, that is, the quantity $C_pTg^{-1}\Delta P/(\sigma T^4)$ where C_p is the heat capacity of air at constant pressure, T is the air temperature, g is the acceleration of gravity, ΔP is the pressure difference between the surface and the 85 kPa pressure level, and σ is the Stefan-Boltzmann constant.

Unlike the situation early on, from about 100 to 120 days after smoke injection the amount of cooling in the CCM/mixed layer model is about as small or smaller than that in Robock's EBM, despite doubled smoke loading. Furthermore, throughout the second half of the CCM simulation land surface temperatures immediately south of the assumed smoke cloud are significantly warmer than control values (see the hatched areas in Fig. 1). The EBM's surface temperatures are everywhere colder when smoke is injected into the model, for the following reason. Since the EBM has no height resolution the method of assuming smoke is to simply decrease the solar constant by the amount corresponding to absorption by the appropriate optical depth. This situation is quite different from that expected in the real atmosphere, in which smoke warmed by absorption of sunlight should overlie a cool surface (see Fig. 1*a* in ref. 8). It is possible that in the CCM simulation some of the heat from the upper troposphere is eventually transported (presumably via infrared radiation[16]) down to the surface, reversing long-term surface cooling in those areas which are not covered by smoke. This effect would no doubt be smaller in a more realistic treatment of protracted nuclear war-generated smoke, which would be more widespread, thinner, less black, and higher than the idealized fixed smoke considered here; nevertheless it is possible that the effect could moderate long-term surface cooling.

Partial support for the foregoing hypothesis comes from an analysis of the GCM's land surface energy budget averaged over the final 10 days of the model run. At 20° N latitude infrared flux from the atmosphere to land surface is 92 W m^{-2} greater in the smoke-perturbed case than in the control case. A smaller warming effect (28 W m^{-2}) is produced by loss of upward latent heat flux due to a drying out of soil moisture in the 15-30° N latitude zone (Fig. 2). The latter type of warming mechanism has been observed in a previous GCM simulation of nuclear war-generated smoke effects[17]. However, underneath the smoke (47° N) the dominant change in surface energy balance is between decreased absorption of solar radiation and decreased upward infrared and sensible heat fluxes; within this latitude band, the infrared flux received by the surface from the atmosphere is not significantly greater in the smoke-perturbed case than in the control case.

The differences in soil moisture between the control and

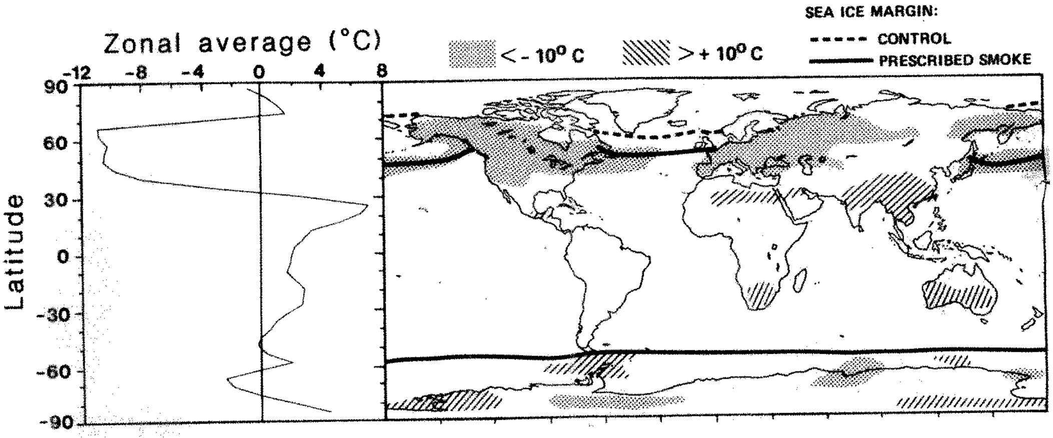

Fig. 3 Surface temperature difference (prescribed smoke case minus control case) averaged over days 110 to 120 after smoke injection. On the left side the zonal average is shown as a function of latitude. On the right side the geographical distribution is shown, with shaded areas indicating greater than 10 °C cooling and hatched areas indicating greater than 10 °C warming; also the control case (thick dashed line) and perturbed case (thick solid line) sea ice margins are indicated (the two coincide in the Southern Hemisphere).

perturbed cases (Fig. 2) are for the most part as expected from previous experiments with the CCM[9]. In general, the CCM responds to fixed upper tropospheric smoke loadings by decreasing cloudiness where the smoke is heated by sunlight while increasing cloudiness at lower levels, resulting in only small changes in precipitation underneath the smoke. (This result is not consistent with results from the Oregan State University two-layer model[17] and may be due to the CCM's assumptions of 100% relative humidity immediately above the surface together with boundary layer condensation at only 80% relative humidity.) Accordingly, soil moisture is greater underneath the smoke in the perturbed case, where surface cooling inhibits evaporation. As pointed out above, soil moisture essentially disappears from latitudes 15-30° N, where surface warming from enhanced downward infrared radiation increases evaporation during the first half of the smoke-perturbed model integration. For latitudes 15-30° S, the expected location of the downward branch of an enhanced cross-equatorial Hadley circulation in the perturbed case (see Fig. 4*b* in ref. 8), a decrease in precipitation leads to the drying out of soil moisture apparent in Fig. 2. Finally, soil moisture more than doubles in the perturbed case in a narrow latitude band centered on 8° N due to a burst of precipitation during the last 10 days of the model integration, which occurs for unknown reasons.

The geographical distribution of surface cooling and sea ice averaged over the final 10 days of the CCM/mixed layer model simulation is depicted in Fig. 3. As expected from Robock's EBM results[7] the extent of sea ice is significantly greater in the smoke-perturbed case. The GCM obtains greatest cooling in the western parts of the North American and Eurasian land masses, downwind of the sea ice, in contrast to early times when maximum cooling occurs in the eastern parts[8]. Cooling of Europe due to enhanced North Atlantic sea ice has a palaeoclimatic analogue: the Younger Dryas period, 10,000-11,000 years ago, exhibited this phenomenon according to both observational[18] and model[19] studies.

In summary, the three-dimensional GCM simulation, while qualitatively confirming a positive feedback due to sea ice that enhances long-term cooling due to nuclear war-generated smoke, reveals additional effects that can moderate or perhaps even reverse long-term cooling. The latter appear to dominate by the end of the GCM run, when the extent of land area that is cooled in excess of 10° C is much smaller than it is in the analogous EBM experiment ('Run 4' in Fig. 1 of ref. 7) despite the fact that the GCM experiment assumed more smoke. However, both classes of effects are probably exaggerated by the assumption of very large quantities of smoke that is fixed in space, and by other model simplifications such as lack of ocean heat transport (leading to overprediction of sea ice). It is therefore necessary to perform more realistic GCM experiments, particularly including model-computed smoke transport and removal, in order to quantitatively assess the relative importance of the positive and negative feedbacks discussed here.

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Trapped radicals in titania gels

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Free radicals are in general very reactive entities capable only of transient existence. Nevertheless, several methods have been devised for their detection and identification, including stabilization by matrix isolation procedures[1] and detection by spectroscopic methods. Radicals can be readily produced by oxidation-reduction processes where electrons are transferred from the solute to the matrix or conversely. Here we report the first production of trapped O_2^- free radicals generated in a titania-gel matrix by the initial reaction of Ti(III) ions with H_2O_2 and the subsequent precipitation with NH_4OH. We also have indications that the detected paramagnetic species may be complexed to the Ti(IV) ions. A striking feature in our results is the very high 2-year stability of these trapped O_2^- radicals. We are able to confirm unequivocally the mechanism proposed by Haber and Weiss to explain the Fenton reagent reactions[2].

It is well known that free radicals are intermediates in many redox processes. The most important radical-forming redox reactions involve a metal ion that can undergo a one-electron transfer. Of these, the reaction of H_2O_2 with Fe(II) ions is the best known. Fenton discovered this reaction in 1894 and in 1932 Haber and Weiss[3] proposed a mechanism that is now generally accepted.

It has been shown[4] that if Fe(II) is replaced by Ti(III) the redox reactions between H_2O_2 and Ti(III) could be, in accordance with the postulated Haber-Weiss mechanism, formulated as:

$$\mathrm{Ti(III)} + \mathrm{H_2O_2} \rightarrow \mathrm{Ti(IV)} + \mathrm{HO^-} + \mathrm{HO^{\bullet}} \qquad (1)$$

$$\mathrm{HO^{\bullet}} + \mathrm{Ti(III)} \rightarrow \mathrm{HO^-} + \mathrm{Ti(IV)} \qquad (2)$$

and at higher H_2O_2: Ti(III) ratios

$$\mathrm{HO^{\bullet}} + \mathrm{H_2O_2} \rightarrow \mathrm{H_2O} + \mathrm{HO_2^{\bullet}} \qquad (3)$$

$$\mathrm{HO_2^{\bullet}} + \mathrm{H_2O_2} \rightarrow \mathrm{O_2} + \mathrm{H_2O} + \mathrm{HO^{\bullet}} \qquad (4)$$

$$\mathrm{HO_2^{\bullet}} \leftrightharpoons \mathrm{O_2^-} + \mathrm{H^+} \qquad (5)$$

According to reaction (5) $HO_2^{\bullet}$ and O_2^- are in equilibrium and in strongly acidic solutions $HO_2^{\bullet}$ should predominate. So far the formation in solution of the species O_2^-, in measurable quantities has not been likely because of the strong acidity of the reaction system, thus there has been no report of detection by electron-spin resonance (ESR) of paramagnetic O_2^- species generated from the redox reactions between Ti(III) ions and H_2O_2 in aqueous solutions. Other investigators[4-6] have detected, by using fast-flow techniques, the generation of $OH^{\bullet}$ and $HO_2^{\bullet}$ radicals in the latter type of reactions. The formation of O_2^-, O_2^+ and O^- species on the surface of TiO_2 was identified only in the case of vacuum-outgassed and oxygen-treated titanium dioxide[7-10].

In this work the titania gels were prepared by adding excess H_2O_2 (30 volumes) to a 15% titanous chloride solution and then adding a 1:1 ammonia solution until the required *p*H was

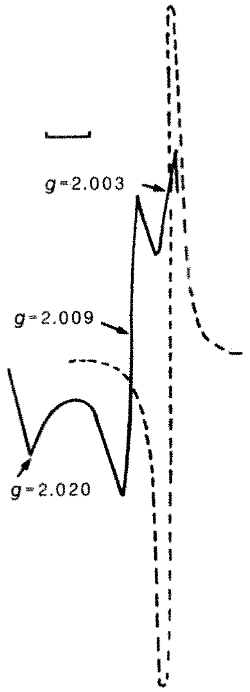
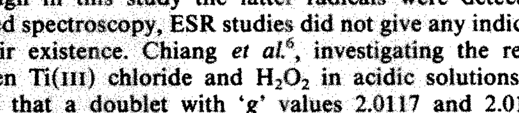


Fig. 1 ESR spectrum of colloid TiO_2 prepared at a pH of 6.9 and aged in distilled water for two years (dotted line, 1,1 diphenylpicrylhydrazyl (DPPH), $g = 2.0036$). Scale bar, 10 gauss.

attained. The gels were then filtered, washed free from chloride and dried in air at ~110 °C for 24 h. Gels prepared under conditions of acidic pH values displayed, at room temperature, by ESR a triplet with 'g' values 2.003, 2.009 and 2.020 (Fig. 1) which, according to the literature[8,10], was identified as belonging to O_2^- on TiO_2. It is interesting that, in this study, the ESR spectrum of such radicals did not escape detection. Indeed, in general for powders at room temperature, large anisotropy broadening occurs and is responsible for undetectable signals.

A very striking observation is that the triplet persisted after keeping the latter gels in air for 2 years. When the batches from which these gels were prepared were aged for 2 years in distilled water then heat-treated for 24 h at ~110 °C in air, the same triplet appeared in the ESR spectrum. It has been shown[11,12] that titania gels, prepared in a manner similar to the present one were formed of large polymeric entities resulting from crosslinking at intervals between these chains:



It therefore seems plausible to assume that it is this network structure that may be responsible for the bulk trapping of the free radicals and for the prevention of recombination.

Infrared studies indicate a broad band at 1,045 cm^{-1} for these gels characteristic of O_2^- radicals on TiO_2 (ref. 13) and a band at 1,389 cm^{-1} characteristic of the $HO_2^\cdot$ radicals[14] (Fig. 2). Although in this study the latter radicals were detected by infrared spectroscopy, ESR studies did not give any indications of their existence. Chiang *et al.*[6], investigating the reaction between Ti(III) chloride and H_2O_2 in acidic solutions, have shown that a doublet with 'g' values 2.0117 and 2.0102, is generally obtained with such systems. They identified the peaks as resulting from complexes involving $OH^\cdot$ and $HO_2^\cdot$ radicals and Ti(IV), respectively, and showed that the formation of other radicals from this system actually took place 'within' the Ti(IV) complex. We did not observe such a doublet in our spectra; but it is possible that the 2.0102 line is superimposed on our observed triplet.

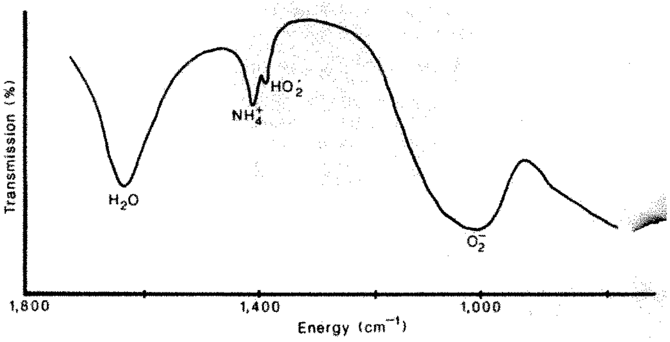


Fig. 2 Infrared spectrum measured on colloidal TiO_2 prepared at a pH of 2.5.

Gels prepared under neutral or alkaline pH values did not indicate, at room temperature in their ESR spectra, the existence of any paramagnetic species, but when the batches from which these gels were prepared were aged for 2 years in water and heat treated for 24 h in air, at ~110 °C the triplet reappeared in the ESR spectrum. Infrared spectra measured on the unaged 'neutral' or 'high-pH' gels displayed peaks at 970 and 1,080 cm^{-1}, which, according to the literature[15], are attributed to bending modes of hydroxy complexes: the latter to a bending mode of a terminal (OH) and the former to a bending mode of a bridging (OH). No band was detected at 1,389 cm^{-1}.

The disappearance of the triplet in the ESR spectrum of these gels is therefore accounted for by assuming that at high pH values, hydroxyl ions would totally replace the O_2^- species in the inner coordination sphere of the Ti ion. The reappearance of the O_2^- species after ageing in water could be explained if, on ageing, these free radicals (presumably near the Ti(IV) ion) diffuse back or regenerate into the inner coordination sphere of the titanium and reform a complex with the Ti(IV) ion. This suggestion is possible considering the non-rigid structure of the titania gel, which implies that the Ti(IV) is a component of the paramagnetic species giving the ESR signal.

Infrared spectra of the water-aged 'neutral' and 'high'-pH titania gels showed also regeneration of the 1,045-cm^{-1} band characteristic of the O_2^- species, but the 1,389-cm^{-1} band did not reappear in the latter case. These results give an unequivocal confirmation of the Haber-Weiss mechanism, according to which, in alkaline or neutral media the O_2^- species should predominate.

Henglein and co-workers[16] developed techniques of probing and using the ability of colloidal TiO_2 to store electrons or holes, which were used to reduce or oxidize compounds in solution. The absorption spectra of the long lived excess of electrons and positive holes trapped at the interface were measured by flash photolysis.

In a series of controlled experiments, titania gels prepared at different pH values from $TiCl_3$ and NH_4OH with oxygen gas as oxidizing agent, preparation described previously[12], did not display by ESR any observable signals, thus providing further evidence that the detected paramagnetic species originate from the redox reaction between H_2O_2 and Ti(III). Also, infrared studies carried out on these gels did not display the 1,389 cm^{-1} and 1,045 cm^{-1} bands.

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Mechanism of diopside dissolution from hydrogen depth profiling

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The basic processes involved at the reaction interface during the aqueous dissolution of crystalline silicates are a matter of controversy. In particular, the existence of a hydrated or protonated surficial layer which has been invoked to explain dissolution kinetics has not been demonstrated yet. By using a resonant nuclear reaction, which allows hydrogen depth profiling, we present, in the case of diopside, the first direct evidence that dissolution proceeds via a surficial hydration over thicknesses of about 1,000 Å. This evidence is combined with X-ray photoelectron spectrometry and secondary ion mass spectrometry data, to show that approximately congruent leaching at energetic portions of the mineral surface allows permeation of the resulting porous structure by water, much of which is strongly bonded to the silicate surface. Thus, we suggest that the migration of molecular water into the surface could be a key step in the dissolution kinetics of diopside and probably of other cationic silicate minerals.

The aqueous dissolution of minerals has been a subject of intensive investigation for more than 25 years because of its importance in Earth sciences. In fact, water-rock interactions have important implications in various fields such as weathering which is a key step in the geochemical cycles of elements such as silicon, aluminium, magnesium and iron, ore forming processes. However, the basic processes involved at the reaction interface, particularly for silicates, are still a matter of controversy. Among the models proposed, essentially two main mechanisms can be distinguished, depending on whether the rate determining step is a transport step, for example, a transport of reactant through an altered surficial layer[1-3] or the dissolution rate is controlled by a surface reaction[4-8]. In this latter theory, the rate limiting step for dissolution has been attributed to the irreversible decomposition of a surficial protonated activated complex, the stoichiometry of which depends to a large extent on the crystallochemical properties of the mineral at the surface active sites[5,7,8]. This theory is now widely accepted, as a chemically altered layer has not been seen on mineral surfaces dissolved in natural or laboratory conditions[5,6,9], either by direct scanning electron microscopy observations or through chemical analyses with X-ray photoelectron spectrometry (XPS). Also, in support of the surface control of reaction rates, recent studies have pinpointed the important role of surface defects (for example, point defects, dislocations) on the dissolution kinetics[6,9,10,11]. However, the existence of a protonated material, at the very surface of the solid[5,10,11], has been postulated on indirect evidence derived from chemical analysis of elements other than hydrogen but has never been directly proved.

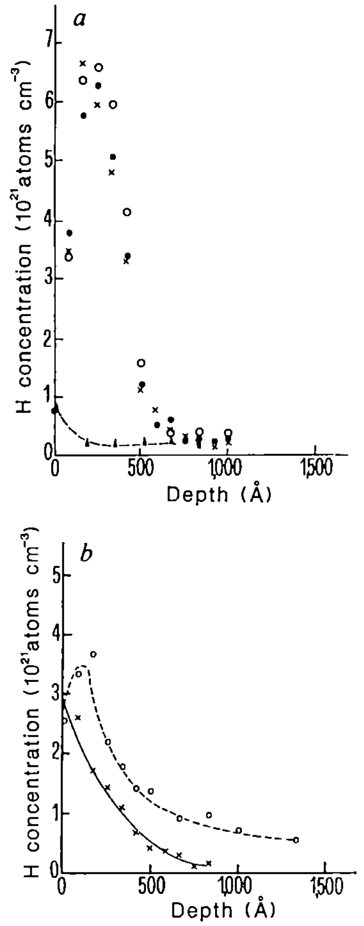

Fig. 1 Evidence for hydrogen penetration in leached diopside. *a* shows RNR H-depth profiles before and after leaching in various conditions ▲, diopside 1 untreated; ○, diopside 1 leached for 75 days at 25 °C and *p*H 2; ●, diopside 1 leached for 75 days at 25 °C and *p*H 6; ×, diopside 2 leached for 75 days at 25 °C and *p*H 2. *b*, The effect of a one hour post-leaching thermal treatment at 150 °C on the RNR H-profile of sample no. 1. ○, Diopside 1 leached; ×, diopside 1 leached and treated for an hour at 150 °C.

In this work, we have applied an analytical technique based on a specific resonant nuclear reaction (RNR) which allows a direct depth profiling of hydrogen on near-surface regions in order to untangle this question. This technique has already been applied successfully to silicate glasses[14,15] and more recently to ion-bombarded minerals[16]. We have chosen diopside because a recent and detailed XPS study[5] has been performed on this mineral, and because there was a reasonable chance of proving whether a hydrated layer was present on the mineral surface during aqueous dissolution. In the first step of this investigation, we concentrated our efforts on the chemical characterization of the mineral surface after dissolution, as it seemed to us important to show unambiguously the presence of a hydrated layer on dissolved diopside.

Centimetre-sized polished sections of two monocrystalline diopsides (sample no. 1 from Rothenkopf (FRG), and sample no. 2 from Val d'Alla (Italy)) were subjected to dissolution in buffered aqueous solutions placed in teflon bottles (*p*H 2 and 6) at 25, 51 and 100 °C during periods ranging from 5 to 75 days, and with a surface area to solution volume ratio $\sim 2\times 10^{-3}$ cm^{-1}. The samples were cut along the c-axis and polished very carefully to produce a flat surface necessary for proper analysis; in this procedure we have tried to minimize defect production by using fine-grained polishing. In addition, to avoid hydration during polishing we used ethanol or ethylene glycol as a paste solvent instead of water. The buffers used in these experiments were mixtures of 0.2 mol l^{-1} KCl and 0.2 mol l^{-1} HCl (pH 2) and of 0.1 mol l^{-1} K-hydrogen phthalate and 0.1 mol l^{-1} NaOH aqueous solutions (pH 6). The chemical analyses of the diopside crystals were performed with a Camebax electron microprobe and are, for samples 1 and 2 respectively: $Ca_{.98}Mg_{.93}Fe_{.07}Si_2O_6$ and $Ca_{.98}Mg_{.97}Fe_{.045}Si_2O_6$. The H-depth profiles were obtained with the $^1H(^{15}N, \alpha\gamma)^{12}C$ nuclear reaction which presents a resonance at 6.385 MeV and allows a resolution of ~30-100 Å. The principles of this technique are presented in ref. 14 and our particular operating conditions are described in ref. 15. Before each run we made the H-profile of a reference allowing a quantitative evaluation of the hydrogen concentration; this reference consists of a monocrystal of silicon implanted with a given dose of 30 keV hydrogen ions. We observed also that the experimental H-profile reproduces perfectly the expected theoretical distribution of implanted hydrogen with the exact range. In order to avoid any charging effect, the samples were covered with a ~100 Å aluminium coating. The results obtained are described below.

Before any dissolution, diopside samples exhibit very limited H penetration (Fig. 1*a*). The H-profile shows a maximum at the very surface $\leq 10^{21}$ H cm^{-3}, that is, ≤ 1 atom %, and extends to a depth <200 Å. A similar profile has been obtained with diopside sample no. 2. The corresponding surficial amount of hydrogen is probably due to a contamination linked to sample preparation, residual hydrocarbons in the vacuum chamber or contact of samples with atmospheric moisture. After dissolution (up to 75 days) by contrast, one notes a marked H penetration in the crystals (Fig. 1*a*). The profiles are almost identical for the two diopsides and the two pHs. The shape of the profiles is quite unexpected as they exhibit a sharp maximum beneath the surface, the maximum hydrogen concentration observed being $\sim 6.6 \times 10^{21}$ H cm^{-3} at around 250 Å (~8.5 atom %) for samples leached for 75 days. Moreover, the hydrogen profile extends to ~700 Å from the surface of the mineral. These H-profiles were reproducible after sample storage up to one year as well as stable under the $^{15}N^+$ beam, even after one half hour bombardment on the same spot.

These results represent the first direct evidence for an important H penetration in a crystalline silicate during its aqueous dissolution. However, our technique does not allow by itself any discrimination between hydrogen containing species (H^+, H_3O^+ or H_2O).

In order to further characterize the chemical form of hydrogen, one sample of diopside no. 1, has been heated for one hour at 150 °C before analysis. The resulting RNR profile (Fig. 1*b*) shows a partial loss of hydrogen of ~25%. This feature indicates that hydrogen is probably partly in the form of molecular water, easily released during thermal treatment, and also, as a species, strongly bonded to the silicate network. This combination of labile and bonded forms of hydrogen could also explain the peculiar peaked shape of the profiles similar to those observed with silicate glasses[17], because the pumping of the sample in the vacuum chamber would have partially removed water from the surface-most zones resulting in a sub-surface maximum. In addition, we have investigated the effect of various parameters on the dissolution characteristics: (1) with increasing temperature, the general trend is that the total amount of penetrating hydrogen decreases (Fig. 2*a*). For example, after 30 days at 51 °C the maximum of hydrogen is $\sim 2 \times 10^{21}$ H cm^{-3} (~3 atom %), although it corresponds to a greater depth ~500 Å, and is even smaller at 100 °C after 8 days of leaching. Such an observation suggests uptake of H_2O as opposed to H^+, with decreasing H_2O uptake with increasing temperature explained by decreasing surface hydration. (2) There is surprisingly no marked pH dependence in the amplitude of H penetration. For instance, diopside no. 1 leached 30 days at 51 °C shows very similar H-profiles at both pH 2 and 6 (Fig. 1*a*). This also tends to support the penetration of hydrogen into the mineral in the form of molecular water even in acidic solutions and not only as H^+ ions replacing cations within the crystal as suggested by the protonated 'surface layer' theories. Similar conclusions have been reached for silicate glasses[17,18]. (3) We have also pre-treated the sample surface with a HF + H_2SO_4 solution at 25 °C. By this process, dislocations or fissures originally present or possibly induced by the polishing and preparation technique[5] are enlarged. With HF treatment we find that H penetration seems to be slightly higher than without any HF treatment, the maximum H-concentration and depth being 2.8×10^{21} H cm^{-3} and 600 Å instead of 1.6×10^{21} H cm^{-3} and 400 Å, respectively (Fig. 2*b*). This is to be expected if the HF treatment produces tiny 'holes' in the surface which are filled with water. In addition, the samples have been carefully examined with both an optical microscope and a SEM and we do not observe any detectable fissuring of the untreated surfaces. Thus the 'holes' result probably from the enlargement of original dislocations by deep etch pit formation.

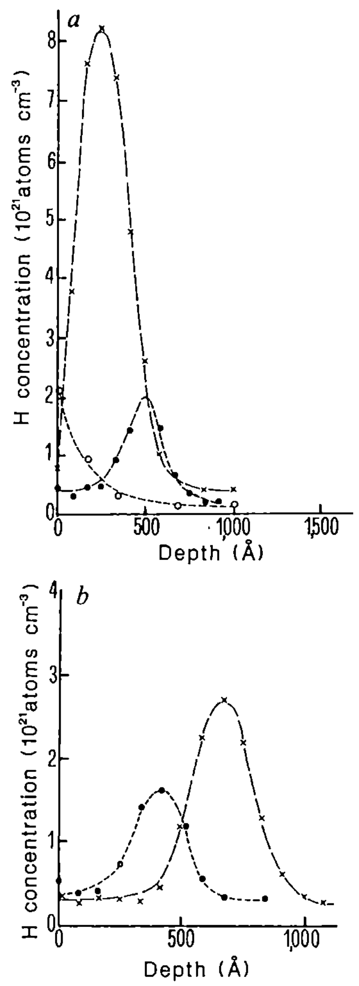

Fig. 2 Panel *a* shows the temperature dependence at pH 6 for sample no. 1 leached, ×, 75 days at 25 °C ●, 30 days at 51 °C and ○, 8 days at 100 °C. *b*, The effect of pre-etching with a HF + H_2SO_4 solution on sample no. 2 subsequently leached, ×, 30 days at 51 °C and pH 2 compared to ●, the reference sample normally leached under the same conditions.

Data on elements other than hydrogen were obtained by XPS and secondary ion mass spectrometry (SIMS) which allow surface analyses and depth profiles of constituent elements of solids. By using XPS, it was found[5,13] that during laboratory dissolution of diopside at pH ~ 2-6 a very thin Ca-depleted zone, on the scale of a few atomic layers (5-15 Å), formed very early on the surface (during the first few hours) but did not increase in thickness as the experiment continued (up to 40 days). This was explained by a stoichiometric dissolution of diopside after a short initial incongruent reaction. Complementary data on elemental depth profiles by SIMS, which will be reported elsewhere, are compatible with XPS results in showing a decrease in the signal of all elements after leaching, along with a preservation of diopside original stoichiometry. This decrease in all elements towards the surface is believed to indicate an increase in the porosity of the surface, the pore space of which, on the basis of RNR results, is filled with water.

There is therefore clear evidence for the presence, during dissolution of diopside, of important quantities of hydrogen probably as H_2O up to a depth of $\sim$1,000 Å coupled with a notable depletion in all elements signifying an increase in porosity over this thickness. Most of this water is strongly bonded within the pores as attested to by its presence after being subjected to high vacuum, ion bombardments and even heating for one hour at 150 °C.

Our results do not agree with the idea that the surface of diopside is represented by a newly-precipitated hydrated phase. Such a precipitate model could indeed explain the existence of a thick hydrogen-containing phase on the surface, but it is incompatible with the H-profiles that we have observed, as this phase should have a constant hydrogen concentration with depth. Also, one would not expect to find essential Ca-Mg-Si stoichiometry as suggested by our XPS data.

Likewise, when one compares the variations with depth of the concentrations obtained by RNR, SIMS and XPS on leached diopside, one concludes that there is no simple correlation between the penetration of hydrogen into this mineral during dissolution and the remobilization of constituent elements. Therefore, our results cannot be accounted for by a simple ionic diffusion or interdiffusion model neither through a leached residual layer or through a reprecipitated one. This result is markedly different from the case of simple silicate glasses for which a ratio between hydrogen and network modifiers $\sim$1 or 3 has been measured, leading investigators[14,15,19] to propose a leach model based on an H^+ (or H_3O^+)-Na^+ (or alkali$^+$) interdiffusion.

The absence of a direct correlation between hydrogen penetration and cation release, as well as the behaviour of leached samples upon heating, also supports the hypothesis of molecular water permeation as already postulated for silicate glasses[17,20]. Diffusion of molecular water and its reaction with the silicate network could henceforth be an important limiting step for the kinetics of silicate dissolution. The density of defects (especially dislocation cores) should play a major role in the migration of water by permitting water molecules to jump from one interstice to another[21]. Simultaneously, the leaching and hydrolysis of the silicate network would 'open the structure' until complete destruction and would enhance the penetration of water inside the mineral. The reaction of water with the network would be responsible for the strong bonding of a fraction of hydrogen and its retention. Our data suggest the formation at the surface of leached diopside of a porous hydrated silicate depleted almost congruently in all elements and therefore of much lighter density. This conclusion is compatible with previous experimental results[5,9] on diopside and other silicates (albite, enstatite, bronzite and tremolite), which indicate essentially congruent dissolution behavior and a stoichiometric composition of solids below the outermost $\sim$10 Å. It is also in agreement with the suggestion of Berner *et al.*[22] that the surface of dissolving silicates is porous because of dissolution along dislocations and microcracks, for example.

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Processes regulating sulphate flux after whole-tree harvesting

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Terrestrial processes that regulate transfer of strong-acid anions (for example SO_4^{2-}, NO_3^-, Cl^-) play an important role in determining the acid–base status of surface waters[1]. Anthropogenic perturbations of forested watersheds can alter these processes, resulting in changes of surface-water chemistry[2,3]. Much controversy has arisen over the relative importance of acidic deposition, natural processes of soil acidification and the effects of changes in land use on the acidification of surface waters[4,5,6]. Forest clearcutting represents a useful experimental tool to evaluate the effects of changes in strong-acid loading on biogeochemical processes controlling SO_4^{2-} retention and release. Here we report that after the whole-tree harvesting of an experimental watershed at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA, increased mineralization and nitrification led to substantial NO_3^- loss, acidification of soil solutions and increased soil adsorption of SO_4^{2-}. As a consequence, solution concentrations and streamwater efflux of SO_4^{2-} declined. Substantial increases in streamwater concentrations of H^+ and potentially toxic inorganic Al^{n+} after removal of biomass also occurred. A similar disruption of the soil N cycle observed in areas of forest decline[7,8] suggests that decreased vegetation uptake of N may adversely affect surface water quality in acid-sensitive regions.

Previous research at the HBEF has demonstrated the importance of ecosystem processes in nutrient retention. After devegetation of watershed 2 (W2) at the HBEF in 1965, a substantial increase in the efflux of NO_3^-, H^+, Al^{n+} and basic cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) occurred with streamflow. In these experi-

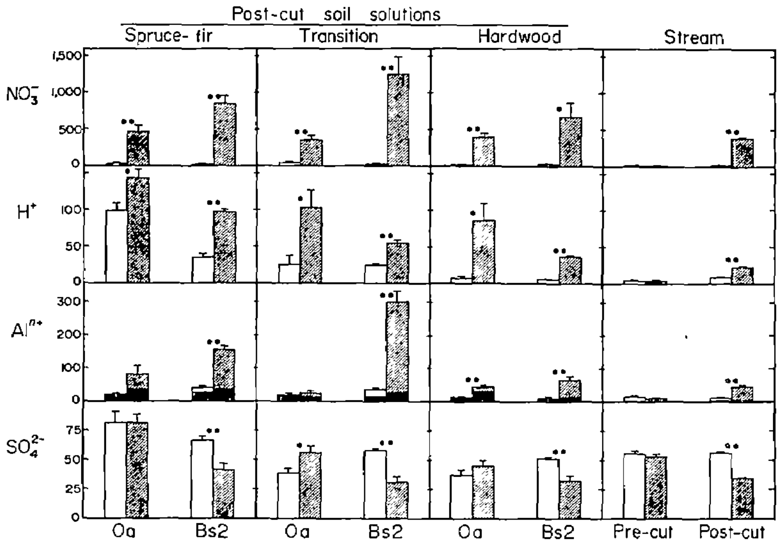

Fig. 1 Soil solution and stream concentrations ($\mu mol\ l^{-1}$) after whole-tree harvesting of watershed 5 (shaded bars) and the reference watershed 6 (clear bars). Darkened (lower) portions of the Al bars represent non-labile monomeric (organically complexed) Al, and the top portion labile monomeric (inorganic) Al[20,21]. Post-cut solutions were collected between 20 November 1984 and 15 March 1985, the dormant period before snowmelt when hydrological fluxes are unaffected by transpiration. Stream solutions before cutting were sampled during the same period in 1983-84, before any clearcut effects. Asterisks denote significant differences between means at the $P < 0.05$ (*) and $P < 0.01$ (**) levels.

ments, conventional commercial clearcutting practices were not used. Rather, trees were felled, left in place, and herbicides applied to prevent vegetation regrowth. These treatments were designed to separate the effects of vegetation uptake from increased decomposition on the mobilization of dissolved nutrients[9,10].

Although acidification and basic cation mobilization were evident, both concentration and efflux of stream SO_4^{2-} declined for the three years of herbicide treatment afterwards. The onset of vegetation regrowth after the cessation of herbicide treatment coincided with a decrease in the acidity of drainage water and an increase in the concentration and transport of SO_4^{2-}. A number of alternative hypotheses could explain the decrease in stream SO_4^{2-} concentrations after devegetation: H1: herbicide application selectively altered soil S transformations; H2: loss of foliar surface area decreased inputs of dry deposition[11]; H3: decomposition of forest-floor organic matter increased, enhancing microbial immobilization of SO_4^{2-} into organic forms[12], or inhibiting organic S mineralization[10]; H4: decreased transpiration resulted in increased hydrological flux and dilution of stream SO_4^{2-} concentrations[10]; H5: sulphate was reduced to gaseous (for example H_2S) or solid (FeS) phases[10]; H6: sulphate was retained owing to precipitation of aluminium hydroxysulphate minerals, such as jurbanite ($AlOHSO_4 \cdot 5H_2O$); H7: adsorption of SO_4^{2-} to free Al and/or Fe oxide surfaces increased owing to acidification of soil solutions[13].

Table 1 Saturation indices (SI) for lower mineral soil (Bs2) and stream solutions from November 1984 to March 1985 in W6 (reference watershed) and W5 (whole-tree harvested watershed)

Site	Watershed	Saturation index	
		Jurbanite	Gibbsite
Spruce-fir*	W6 soil	−0.99	−0.40
(750 m)	W6 stream	−1.38	−1.54
	W5 soil	−0.78	−0.85
	W5 stream	−0.99	0.20
Transition	W6 soil	−0.75	0.14
(730 m)	W6 stream	−0.78	−0.11
	W5 soil	−0.58	0.17
	W5 stream	−0.79	−0.51
Hardwood	W6 soil	−1.87	0.54
(600 m)	W6 stream	−1.13	0.28
	W5 soil	−0.87	0.01
	W5 stream	−0.82	0.21

$SI = \log(Q_p/K_p)$, where Q_p is the ion activity product and K_p is the equilibrium constant. Positive values indicate oversaturation, negative values undersaturation with respect to the mineral phase of interest.

*Spruce-fir soil and stream solutions are typically undersaturated with respect to all mineral phases, owing to a combination of shallow soils (short hydrologic residence time), low pH and high dissolved organic C concentration, all of which favour a kinetic restraint on attaining saturation.

To test these hypotheses, a watershed manipulation experiment was done at the HBEF. From autumn 1983 through spring 1984 an experimental watershed (W5) underwent a commercial whole-tree harvest involving the removal of all aboveground tree biomass. This manipulation was evaluated using the paired watershed approach, in which the experimental watershed and an adjacent untreated reference watershed (W6) were monitored. Both watersheds were instrumented with stream gauging weirs, bulk precipitation and throughfall collectors, and 'tension-free' soil lysimeters[14] beneath the Oa (forest floor) and within Bs2 horizons above impervious schist bedrock. Watersheds 5 and 6 are dominated largely by northern hardwood forest vegetation, consisting of yellow birth (*Betula alleghaniensis*), American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*). A spruce-fir zone at the top of each watershed is dominated by red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*). Elevational differences in vegetation and soil depth contribute to substantial spatial variability in the chemistry of drainage waters in these catchments[15,16]. Consequently, replicate lysimeters were installed in a low-elevation hardwood zone (600 m; $n = 3$) near the base of each watershed, in a hardwood transition zone (730 m; $n = 3$) and in a high-elevation spruce-fir zone (750 m; n-2) near the top of each watershed.

After whole-tree harvesting of W5, patterns of element concentration and transport (Fig. 1) were similar to those after devegetation of W2. Increased concentrations of NO_3^- were released from soil to stream solutions owing to reduced vegetative uptake coupled with increased mineralization and nitrification caused by higher soil moisture and temperature[7,17]. Mineralization and nitrification of soil organic N produces one mole of H^+ for each mole of NO_3^-; however, increases in H^+ concentration were not stoichiometric with respect to NO_3^- release. Rather, increased concentrations of basic cations and Al were also leached from both organic and mineral soil horizons owing to a combination of mineralization, exchange and dissolution reactions. Increases in Al concentration largely occurred as the labile (inorganic) monomeric form, which has been shown to be toxic to aquatic organisms[18,19]. Concentrations of SO_4^{2-} declined significantly ($P < 0.01$) after the whole-tree harvest (Fig. 1) in both lower-mineral soil solutions (W5, Bs2) and streamwater. Beneath the forest floor (Oa), however, SO_4^{2-} concentrations remained stable or significantly increased after the manipulation.

Patterns of solute release enabled us to test the research

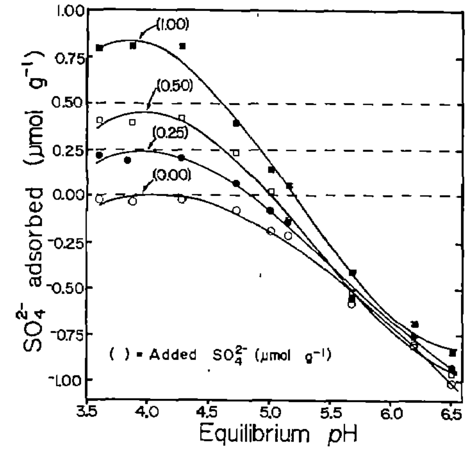

Fig. 2 Sulphate adsorbed by HBEF Bs2 horizon soil at different concentrations of added sulphate across a range of equilibrium solution pHs. Negative adsorption numbers are due to desorption of ambient sulphate. Lines represent the best fit of a third-order polynomial equation.

hypotheses (H1-H7) posed to explain decreases in the SO_4^{2-} concentration of drainage water after forest removal. As herbicides were not applied in the experiment, a selective action of the herbicide on S transformations was not possible (H1). Decreases in dry deposition inputs to the canopy as a reult of canopy loss (H2), increases or decreases in S immobilization or mineralization rates, respectively, in the forest floor (H3), and dilution of SO_4^{2-} due to increased hydrologic flux (H4) would all be expected to result in decreased concentrations of SO_4^{2-} in Oa-horizon leachates. Consequently these hypotheses can be rejected as the proximate cause of decreased SO_4^{2-} concentrations in mineral soil and stream solutions after clearcutting. Decreases in SO_4^{2-} concentration due to reduced dry deposition of S were probably compensated for by increased mineralization of organic S to SO_4^{2-} in the forest floor[12]. Microbial reduction of SO_4^{2-} to S^{2-} (H5) is also unlikely, owing to the high concentrations of NO_3^- in soil solution and the fact that of NO_3^- reduction is more energetically favourable[22] and would proceed before SO_4^{2-} reduction.

Possible control of solution SO_4^{2-} concentrations by jurbanite ($Al(OH)SO_4 \cdot 5H_2O$) precipitation (H6) was investigated by calculating mineral saturation indices (Table 1) for jurbanite and natural gibbsite using the chemical equilibrium model ALCHEMI[23]. This computer model calculates the distribution of inorganic Al complexes, adjusting for temperature and ionic strength effects[23]. Soil and stream solutions were undersaturated with respect to the solubility of jurbanite in both watersheds at all elevations (Table 1), suggesting that jurbanite precipitation was not thermodynamically favourable and probably not responsible for the decline in solution SO_4^{2-} concentrations after the whole-tree harvest. Soil and stream solutions in the transition and low-elevation zones were closer to saturation with respect to the solubility of natural gibbsite, or a mineral of similar solubility. Although the solubility of $Al(OH)SO_4 \cdot 5H_2O$ apparently does not control SO_4^{2-} concentrations at the HBEF, this process is considered important in regions with high atmospheric inputs of SO_4^{2-}, such as parts of the Federal Republic of Germany and The Netherlands[24,25].

Adsorption of SO_4^{2-} onto free sesquioxide surfaces (H7) was investigated using batch titration studies in which Na_2SO_4 was added to suspensions of Bs2 mineral horizon soil (taken from 30 soil pits in W5) with solution pH adjusted between 3.5 and 5.5 with HNO_3 or NaOH. After centrifugation, pH and soluble SO_4^{2-} were measured and adsorbed SO_4^{2-} was calculated from the amount removed from or added to solution. Acidification of the soil solution greatly enhanced retention of SO_4^{2-} (Fig. 2). In the range of pH values experienced by lower mineral soil solutions at the HBEF (4.5 to 5.3), SO_4^{2-} adsorption is highly dependent on pH[26]. As pH decreases to 4.3, the extent of SO_4^{2-} adsorption increases owing to protonation of variable-charge surfaces such as $Al(OH)_3$ or $Fe(OH)_3$. Below pH 4.3, the extent of SO_4^{2-} adsorption diminishes. We attribute this decrease to dissolution of soil Al, which forms soluble $Al\text{-}SO_4^+$ complexes which compete with the adsorbent phase for SO_4^{2-}.

On the basis of laboratory and field soil solution monitoring studies, all of the hypotheses advanced to explain the reductions in stream SO_4^{2-} concentrations after whole-tree harvesting can be rejected with the exception of acidification-induced SO_4^{2-} adsorption in lower mineral soil horizons. Furthermore, adsorption appears to be an important factor regulating seasonal variability in stream SO_4^{2-} concentrations in undisturbed watersheds[27]. Regression analysis of stream SO_4^{2-} and H^+ concentrations (μ equiv. l^{-1}) in the reference watershed over a 2.5 year period yielded a significant inverse relationship ($[SO_4^{2-}] = 145 - 3.25\ [H^+]$; $r^2 = 0.49$; $P < 0.0001$; $n = 31$), consistent with the pH-dependent SO_4^{2-} adsorption hypothesis.

Current models developed to simulate the response of forest ecosystems to strong-acid inputs generally fail to consider pH-dependent SO_4^{2-} adsorption[28-31]. Results of this whole-tree harvest experiment indicate that strong-acid inputs, even though they may change soil solution pH only slightly, can facilitate SO_4^{2-} retention, which buffers surface water acidification. Therefore simulation models that fail to account for pH-dependent SO_4^{2-} adsorption may overestimate the consequences of increased inputs of strong acids to waters draining forest ecosystems.

The whole-tree harvest experiment also demonstrated that disruption of the soil N cycle resulting from biomass removal can cause short-term acidification of soil solutions and streams and the release of toxic inorganic Al to surface waters[18]. In view of current concerns about forest decline[32], a link between forest degradation and surface-water acidification is especially noteworthy. If forest decline is accompanied by substantial NO_3^- efflux, as has been observed in the Federal Republic of Germany[7] and Czechoslovakia[8], then the potential for damage to surface-water quality is present.

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High rates of consumption of bacteria by pelagic ciliates

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It has been thought that ciliate protozoans that consume bacteria are not found in open waters except at micro-sites of high bacterial density[1,2]. Previous laboratory studies, using ciliates isolated from benthic habitats have suggested that (1), ciliates that can ingest bacterial-sized particles have cell-specific clearance rates too low to obtain sufficient food for growth at the bacterial abundances found in most marine and fresh waters[3,4], and (2), spirotrichous ciliates cannot ingest particles of less than 1–2 μm in diameter[3,4]. New data on uptake rates of pelagic ciliates at low bacterial concentrations refute both of these ideas. We and others have found that ciliates, including spirotrichous forms, present in the plankton of both coastal marine waters and lakes can be voracious consumers of bacteria[5–8]. Using a new method for preparing monodisperse, fluorescently labelled 'marker' bacteria, we have measured bacterial clearance rates for pelagic ciliates which are 10–100 times greater than earlier estimates. In waters of a salt marsh estuary, ciliate grazing can account for 100% of estimated protozoan bacterivory. Ciliate bacterivory may 'short-circuit' the microbial loop in aquatic ecosystems by making bacterioplankton production available to metazoan grazers via a simple, two-step food chain.

Water samples containing natural assemblages of ciliates were collected in the Duplin River, a salt marsh tidal embayment on the Georgia, US coast. Ciliates were not taxonomically identified, except for one scuticociliate, *Uronema marina*, which we cultured on a wheat grain infusion. However, based on cell size, ciliature, and number and shape of cell nuclei, we were able to distinguish nine separate morphological types in our samples: four spirotrichs (class Spirotrichea, subclass Choreotrichia)[9], four scuticociliates, including *U. marina* (class Oligohymenophorea, subclass Hymenostomatida)[9], and one free-swimming, 30-40 μm peritrich (class Oligohymenophorea, subclass Peritrichia, order Mobilida)[9]. The spirotrichs and scuticociliates were generally <20 μm in size and numerically dominated the assemblage of pelagic ciliates, as previously noted for these coastal waters[7].

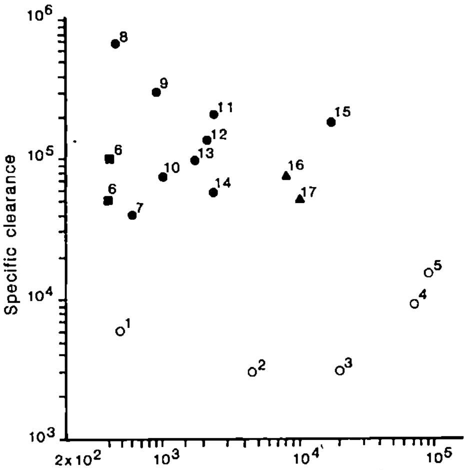

Fig. 1 Previous (open circles) and recent (solid symbols) cell-specific clearance rates (body volumes h^{-1}) determined for pelagic ciliates as a function of ciliate cell volume. 1, *Cyclidium glaucoma*; 2, *Glaucoma scintillans*; 3, *Colpidium campylum*; 4, *Paramecium trichium*; 5, *P. caudatum* (○)[4]; 6, *Cyclidium* sp. (■) (M. Pace and M. Bailiff, manuscript in preparation); 7, *Uronema marina*; 8, Spirotrich a; 9, Spirotrich b; 10, Spirotrich c; 11, Spirotrich d; 12, Scuticociliate b; 13 Scuticociliate c; 14, Scuticociliate d; 15, Free-swimming peritrich (●; this study); 16, *Epistylis rotans*; 17, *Strombidium* sp. (▲; ref. 6—note that the specific clearance rates calculated by Borsheim were in error, too low by a factor of 10^3).
Methods. Experiments were carried out at 25 °C, approximately *in situ* temperature, in 400 ml Whirl-pak bags, presoaked in 10% HCl and copiously rinsed with deionized water. Short-term uptake of bacteria by ciliates present in the water was evaluated using preparations of a 0.6-μm (0.10 μm^3), gram-negative bacterium, isolated from local waters, which had been simultaneously heat-killed and stained with the flurochrome 5-(4,6-dichlorotriazin-2-yl)-amino-fluorescein (DTAF) (B.F.S. *et al.*, manuscript in preparation). The fluorescently labelled bacteria (FLB) are not toxic to ciliates, and can be metabolized to support rapid ciliate growth (B. Sherr *et al.*, manuscript in preparation). FLB were added to samples of estuarine water at concentrations of 1-4×10^5 ml^{-1}, which ranged from 3-5% of the natural bacterioplankton abundance, determined before initiating the experiment by acridine orange (AO) direct counting[24]. After addition of FLB, 5 ml subsamples were removed from the bags and fixed with a final concentration of 2% borate-buffered formalin at time zero and at 5-min intervals for periods of 15-30 min. Time zero subsamples were examined for abundance of FLB by filtering 1.0 ml aliquots onto irgalan-black stained, 0.2 μm Nucleopore filters. A Zeiss Universal epifluorescence microscope outfitted with a 75-W xenon lamp was used for enumerating bacteria at 1250×, using an AO filter set (Zeis 47 77 09); with this filter set the FLB fluoresced a bright apple-green. The average number of FLB ingested by each ciliate species with time was determined by filtering the time series subsamples onto 0.8-μm, black-stained Nuclepore filters, staining with the fluorochrome diamidinophenylindole (DAPI)[25], and examining the prepared filters by epifluorescence microscopy. Ciliates were found on the filter at a low magnification (×160) using a DAPI filter set (Zeiss 47 77 02); then the number of FLB within an individual ciliate was counted under oil immersion at ×1,250 using the AO filter set. The rate of uptake of FLB by ciliates was generally linear for 10-20 min, after which the average number of FLB per cell levelled off as digestion of the bacteria approximated the rate of ingestion. We do not know whether the ciliates might have preferentially ingested FLB; however, addition of only trace amounts of FLB to the total population of suspended bacteria probably minimized this potential problem.

Specific clearance rates based on uptake rates of fluorescently labelled marker bacteria (FLB) for the nine ciliate types, expressed as the number of body volumes cleared per cell per unit time, ranged from 4×10^4 h^{-1} to 6.8×10^5 h^{-1} (Fig. 1). These rates are 1-2 orders of magnitude higher than the previous specific clearance rates estimated for ciliates feeding on bacteria[4]. We also calculated, from data reported in two other recent studies in which fluorescent microspheres were used to evaluate ingestion of bacterial-sized particles by a marine *Cyclidium* sp (M. Pace and M. Bailiff, manuscript in preparation) and by two freshwater spirotrichs[6], specific clearance rates 10-20-fold higher than the earlier estimates (Fig. 1). In several of our own experiments we included a separate treatment in which 0.5-μm fluorescent latex microspheres (Polysciences, Inc.) were added at approximately the same concentration as the FLB (B.F.S. *et al.*, manuscript in preparation). We found that the scuticociliates and the peritrich species ingested the microspheres and FLB at equivalent rates; however, the spirotrichs consistently ingested the FLB at a rate 5-fold higher than for the microspheres.

Table 1 Rate of ciliate consumption of bacteria

Date	Bacterioplankton abundance at T_0 (10^6 cells ml^{-1})	Bacterivory (10^5 cells consumed ml^{-1} h^{-1}) Total consumption	Ciliate consumption
7 June 1985	8.4	1.7	1.9
1 July 1985	6.9	1.8	2.4
16 September 1985	5.5	0.6	1.2
23 September 1985	3.8	0.3	0.2

Comparison of estimates of total bacterivory by <17-μm estuarine protozoa using prokaryotic inhibitors to stop bacterial growth[17], with estimates of ciliate bacterivory calculated from the population abundance of <17-μm ciliates determined for each experiment and the ciliate clearance rates measured here.

The highest clearance rate in our study was found for a 8-10 μm diameter spirotrich (Fig. 1). We calculated that if this ciliate fed continuously at such a rate, it would be able to ingest sufficient biomass to sustain one doubling per day, assuming a 33% gross growth efficiency, at a bacterioplankton concentration of 15 μg carbon per litre. In our estuarine waters, where the average bacterial cell size is about 0.08 μm^3 (ref. 10), this biomass would equal 8.5×10^5 cells ml^{-1}, using a conversion factor of 0.22 pgC μm^{-3} bacterial biovolume[11]. The idea that pelagic ciliates can grow at the expense of such a low bacterial abundance is supported by the finding of Rivier *et al.*[8] that a 20-30 μm spirotrich, *Strombidium sulcatum*, isolated from oligotrophic waters of the northwestern Mediterranean, grew in laboratory cultures on $3\text{-}6 \times 10^5$ bacteria per ml. The other ciliates examined in the present study (Fig. 1) would require a higher density of bacteria to sustain one doubling per day, up to 170 μgC l^{-1}, or 9.6×10^6 cells ml^{-1}. This range of bacterial abundance, 15-170 μgC l^{-1}, is typical of coastal marine waters[12,13], and also of freshwater systems[14-16].

We have previously determined rates of bacterivory by phagotrophic protozoa in 17-μm-screened estuarine water using a selective inhibitor approach[17]. Based on the clearance rates found in the present study, we calculated the potential contribution of the <17 μm ciliates counted in prokaryotic-inhibited samples to the total protozoan bacterivory determined for four summer experiments. We used an average per cell clearance rate (in this case volume of water cleared cell^{-1} h^{-1}) of 140 nl h^{-1} for scuticociliates and 270 nl h^{-1} for spirotrichs. Comparison of the rates of bacterial loss determined in inhibited treatments with the calculated rates of ciliate bacterivory (Table 1) shows that ciliate grazing could account for virtually all of the decrease in bacteria observed in each experiment.

The FLB method we developed to study instantaneous protozoan bacterivory *in situ* is a significant advance over the use of fluorescent microspheres[6], as bacterivorous spirotrichs apparently discriminate against inert particles. Our results indicate that, at least in estuarine waters, there can be a quantitatively significant trophic pathway between bacteria and ciliates in the pelagic food web. This has important implications for the present controversy regarding the contribution of microbial production to metazoan food chains. Ciliates are readily consumed by metazooplankton, including micro-crustaceans and fish larvae[18,19]. Therefore ciliate bacterivory can by-pass at least one step in the bacteria to flagellate to ciliate microbial loop[20], which is currently believed to be a sink rather than a link for carbon flow in pelagic ecosystems[21,22], and make a substantial fraction of the often considerable biomass production of bacteria available to higher order consumers in the food web. As pelagic ciliates are abundant in many parts of the world ocean[7,12], as well as in lakes[23], their role as bacterial grazers is in urgent need of reevaluation.

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Regeneration by supernumerary axons with synaptic terminals in spinal motoneurons of cats

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Axons in the central nervous system (CNS) of mammals do not normally regrow if they are cut, which severely limits restoration of function after injury. We have studied the reactions of adult cat spinal α-motoneurons after chronic transection of their axons in the periphery by labelling single cells with horseradish peroxidase. Twelve weeks after the operation, about a third of the axotomized cells had developed a 'supernumerary' axon originating from the cell-body region. These supernumerary axons had variable trajectories and termination fields in the ipsilateral spinal cord but generally anomalous projections. Ultrastructural examination shows that they give rise to boutons that form morphologically normal synaptic contacts with neuronal profiles, although they contain dense-cored vesicles not normally seen[1,2] in central terminals of α-motor axons. We conclude that axotomized neurons in the mammalian CNS may be able to form new synaptic contacts by means of supernumerary axons in the absence of local damage.

The medial gastrocnemius (MG) nerve of adult cats was transected in the left popliteal fossa. The central stump was ligated and prevented from reinnervating the muscle. Twelve weeks later, glass micropipettes filled with a 25% solution of horseradish peroxidase (HRP; Sigma, type VI) in 0.1 M NaOH and 0.5 m KCl were used for iontophoretic injection of HRP intracellularly into single chronically axotomized MG α-motoneurons[3-8]. The motoneurons were identified by the anti-

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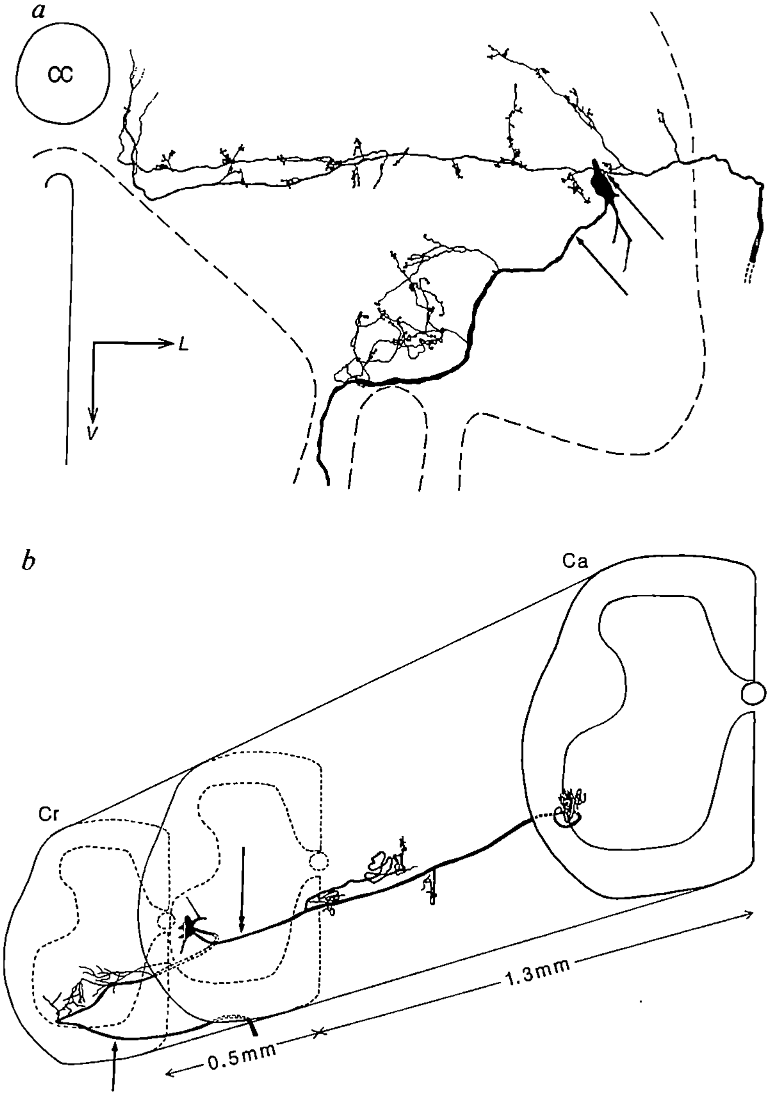


Fig. 1 *a*, Reconstruction in the transverse plane of the axonal systems of a chronically axotomized MG α-motoneuron after intracellular labelling with HRP. The original stem motor axon (single arrow) and the supernumerary axon (double arrow) had separate origins from the cell soma (see Fig. 2). The original stem axon exhibited a conventional ventromedial course and left the spinal cord by the ventral root. It gave rise to three axon collateral systems in the grey matter. The supernumerary axon divided early into two main branches, which elongated in opposite directions. The medial branch extended towards the central canal (CC) and gave rise to multiple thin collateral branches, which terminated in clusters of small bouton-like swellings. The lateral branch entered the lateral funiculus, where it ascended beyond the end of the section series (>920 μm rostrally to the cell soma). It produced two collateral branches within the grey matter. Broken line, border between the spinal grey and white matter; L, lateral direction; V, ventral. *b*, Schematic reconstruction of a portion of the lumbosacral spinal cord together with the intramedullary axonal systems of a chronically axotomized MG α-motoneuron after intracellular labelling with HRP. The original stem motor axon (single arrow) and the supernumerary axon (double arrow) had a common origin from the cell body (see Fig. 3). The original axon gave rise to four axon collateral systems in the grey matter and left the spinal cord through the ventral root. The supernumerary axon descended in the ventrolateral grey matter and terminated 1.3 mm caudally to the cell body. It exhibited few collateral branches, all of unusually large diameter. The collaterals formed tangles and possessed few bouton-like specializations. Ca, caudal; Cr, cranial.

dromic action potentials evoked by stimulation of the central stump of the cut MG nerve. After glutaraldehyde fixation, serial sectioning and histological processing of the spinal cord tissue[3–8], light microscopic reconstructions and ultrastructural analyses of the HRP-labelled axotomized MG α-motoneurons were performed.

Twenty-six chronically axotomized MG α-motoneurons in six cats were studied. In each, the original stem motoraxon still remained (Fig. 1 *a* and *b*, single arrows) and generally gave off one or more axon collateral systems in the ventral grey matter before leaving the spinal cord through the L7 or S1 ventral root. The intramedullary trajectories and projection areas of these original axons and axon collateral systems were broadly similar to those observed in normal non-axotomized α-motoneurons[1–5].

In addition to the original stem motoraxon, eight of the axotomized α-motoneurons in four cats were found to possess an intramedullary supernumerary axon (Fig. 1*a* and *b*, double arrows). Such supernumerary axons have previously not been observed in extensive studies on normal HRP-stained triceps surae[3–9] or phrenic[10,11] α-motoneurons of adult cats. We therefore concluded that the appearance of supernumerary axons in the present axotomized neurons was related to the chronic nerve injury.

The supernumerary axons originated from the cell-body region of the axotomized cells (Figs 1, 2 and 3) and usually exhibited a fairly uniform diameter along their length; no more than one supernumerary axon was detected per axotomized MG α-motoneuron. All supernumerary axons were myelinated (Figs 2 and 3), but the degree of myelination varied from the normal appearance with regular nodes of Ranvier to a more patchy and irregular distribution of myelin along the axon. All the supernumerary axons gave off collateral branches, which generally formed bouton-like specializations of both *en passant* and terminal types (Fig. 1*a*). The supernumerary axons demonstrated a considerable variation in their trajectories and areas of termination. Compared with the situation in normal animals[1–5] the projections were generally anomalous. In this material, the supernumerary axons projected ventrolaterally within Rexed's[12] lamina IX, ventromedially into laminae VII and VIII, medially towards the central canal (Fig. 1*a*) or dorsally into laminae I–IV. In some cases they passed within the ventral or lateral funiculi for varying distances (Fig. 1*a*, *b*). However, they were never seen to extend into the ventral or dorsal roots or to leave the spinal cord elsewhere, nor did they cross over to the contralateral side of the spinal cord.

The ultrastructure of 14 bouton-like specializations originating from the supernumerary axons of two axotomized MG α-motoneurones was examined with the electron microscope. All these specializations were found to contact neuronal profiles and they contained mitochondria as well as numerous dense-cored, spheroid vesicles of 35–65 nm diameter (Fig. 4). The latter observation was unexpected, because intramedullary boutons of normal triceps surae motoraxon collaterals contain only clear, spheroid synaptic vesicles[1,2]. In most of the supernumerary

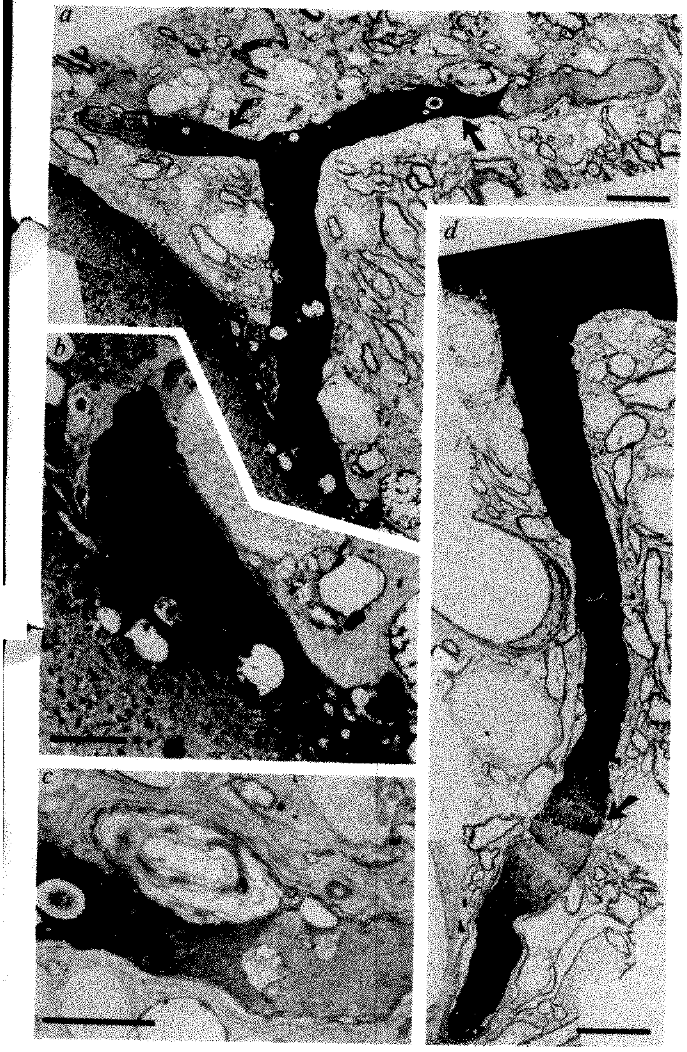


Fig. 2 Electron micrographs of the intracellularly HRP-labelled and chronically axotomized MG α-motoneuron illustrated in Fig. 1*a*. *a*, Photomontage showing the supernumerary axon originating from the cell body and dividing into a lateral (right) and medial (left) branch. Arrows, beginnings of the thin myelin sheaths surrounding the two axonal branches; calibration bar, 5 μm. *b*, The cell-body origin of the supernumerary axon shown at higher magnification; calibration bar, 2.5 μm. *c*, Start of the myelin sheath surrounding the lateral branch of the supernumerary axon shown at higher magnification; calibration bar, 3 μm. *d*, Photomontage showing the original stem motor axon leaving the cell body (top) at another level of the section series. Arrow, beginning of the myelin sheath; calibration bar, 8 μm.

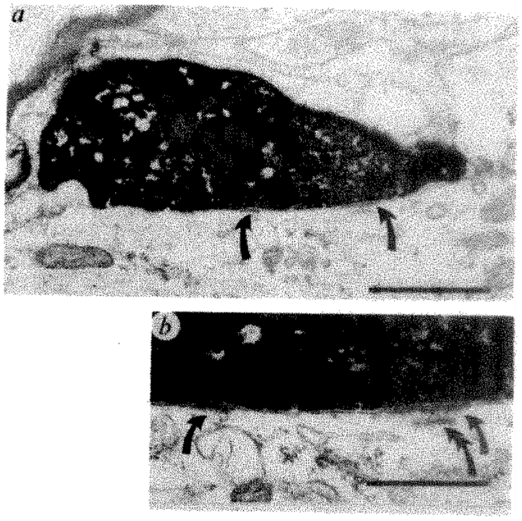
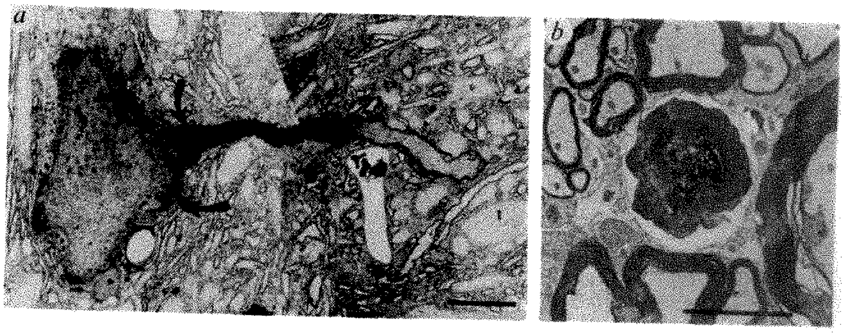


Fig. 4 *a*, Electron micrograph of an HRP-labelled bouton originating from the supernumerary axonal system illustrated in Fig. 1*a*. The bouton exhibits mitochondria, many dense-cored vesicles and a neuronal target of apposition. Arrows, synaptic contact zones; calibration bar, 1 μm. *b*, Synaptic contact zones shown at higher magnification. The synaptic clefts contain dense material and there is also dense cytoplasmic material located at the postsynaptic membrane (arrows). A ribbon of subjunctional dense material (double arrow) is associated with the right contact zone. Calibration bar, 0.5 μm.

boutons the synaptic character was further emphasized by the presence of synaptic clefts with postsynaptic dense material (Fig. 4). Presynaptic densities would not be identified owing to the presence of the intracellular HRP reaction product.

Regenerative elongation of axons or axon-like structures in the central nervous system of mammals has previously been demonstrated in association with central injuries involving the formation of glial or mesodermal scar tissue[13,14]. However, the myelinated processes of uniform diameter that originate from distal dendritic branches after intramedullary axotomy of cat α-motoneurons[14] do not exhibit intramedullary collaterals or bouton-like structures. Furthermore, only dendrites in close proximity to the site of spinal injury were seen to generate such 'dendraxons', all of which elongated in the direction of the scar tissue, so the possibility remains that only lesioned dendrites may have developed the 'dendraxons' or that some trophic influence of the scar tissue may have triggered the 'axonalization' of adjacent dendrites.

Fig. 3 Electron micrographs of the intracellularly HRP-labelled and chronically axotomized MG α-motoneuron illustrated in Fig. 1*b*. *a*, Photomontage showing the common starting point of the original stem motor axon (single arrow) and the supernumerary axon (double arrow) from the cell body. The supernumerary axon had a straight caudal direction from its origin, which explains its lack of extension in the transverse section plane. Calibration bar, 15 μm. *b*, The supernumerary axon located in the lateral white column of the spinal cord about 1.2 mm caudally to the level of the parent cell body. The axon is myelinated and contains HRP reaction product. Calibration bar, 3 μm.



Several investigators have also demonstrated elongation of central axons into peripheral nerve tissue grafted into the mammalian brain or spinal cord[15-20]. This extension or sprouting of central axons is assumed to be associated with trophic factors within the grafted peripheral nerve tissue. The present morphological study demonstrates that regenerative axonal growth within the adult mammalian central nervous system may involve the formation of supernumerary axons originating from the cell body region, and that this regenerative growth can take place in the absence of local changes in the neuroglial environment caused by scar tissue or peripheral nerve grafts. Furthermore, the supernumerary axons are capable of generating boutons, which according to ultrastructural criteria form regular synaptic contacts with neuronal structures. A previously undescribed form of regenerative synaptogenesis in the central nervous system is therefore suggested.

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Stimulation of protein kinase C recruits covert calcium channels in *Aplysia* bag cell neurons

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The modulation of voltage-activated calcium currents by protein kinases provides excitable cells with a mechanism for regulating their electrical behaviour[1-7]. At the single channel level, modulation of calcium current has, to date, been characterized only in cardiac muscle, where β-adrenergic agonists, acting through cyclic AMP-dependent protein kinase, enhance the calcium current by increasing channel availability and opening[3,8-12]. We now report that enhancement of calcium current in the peptidergic bag cell neurons of *Aplysia* by protein kinase C (ref. 13) occurs through a different mechanism, the recruitment of a previously covert class of calcium channel. Under control conditions, bag cell neurons contain only one class of voltage-activated calcium channel with a conductance of ~12 pS. After exposure to agents that activate protein kinase C, these neurons also express a second class of calcium channel with a different unitary conductance (~24 pS) that is never seen in untreated cells.

The function of the neurosecretory bag cell neurons is to initiate a sequence of reproductive behaviours. Activation of these cells leads to major changes in their electrical properties, controlled in part by activation of protein kinase C[7]. This calcium-, phospholipid-, diacylglycerol-dependent enzyme is present in high concentrations in the nervous system of *Aplysia*[14] as well as in mammalian brain[15]. In intact cells, the enzyme may be activated by phorbol esters such as TPA (12-*O*-tetradecanoyl-phorbol-13-acetate; PMA) or synthetic diacylglycerols such as DOG (1,2-dioctanoyl glycerol). In isolated bag cell neurons, injection of purified protein kinase C, or application of TPA or synthetic diacylglycerols enhances evoked action potentials[13]. In whole-cell voltage clamp experiments, TPA pretreatment increases the magnitude of the calcium current although voltage-activated potassium currents are unaffected[13].

Table 1 Unitary Ca channel activity in the absence and presence of activators of protein kinase C

	No. of patches	No channels (%)	Small channels (%)	Large channels (%)
Control	33	64	36	0
TPA pretreatment (10-20 nM)	28	43	36	36
TPA pretreatment (100-200 nM)	6	0	67	50
DOG pretreatment (1.5 μg ml^{-1})	3	0	33	66
TPA applied after patch formation	11	55	45	0
TPA in pipette	8	50	50	0

Recording conditions as described in Fig. 1 legend unless otherwise noted. Inward channels were classified as small (slope conductance <16 pS) or large (>20 pS). Cells were pretreated with TPA for 10-120 min, or with DOG for 14-40 min. Shorter pretreatments were not attempted because enhancement of spike height by TPA requires 2-5 min in cells impaled with microelectrodes[13]. In experiments in which TPA was included in the recording pipette or added to the bath after formation of the seal, patches were monitored for 10-60 min with no apparent large channel activity. The apparent increase in the number of small channels by very high doses of TPA (line 3) was not statistically significant (Fisher's exact test, $P = 0.10$). We did not attempt many experiments under these conditions because such doses of TPA are sometimes seen to induce spontaneous activity in cells impaled with microelectrodes.

We used patch clamp recording techniques[16] to study the mechanism of the enhanced Ca current at the single channel level. Cell-attached patch recordings were made on bag cell neurons maintained in primary culture, using pipettes containing 185 mM Ba^{2+} rather than Ca^{2+} as the charge carrier to facilitate resolution of unitary calcium channel currents[8]. In normally polarized cells, depolarization of the membrane patch 20-30 mV above the resting potential evoked inward unitary current events such as those shown in Fig. 1*a*. This type of activity was characterized by a slope conductance of <16 pS, and was routinely observed in both control patches and patches on cells pretreated with 10 nM TPA (Table 1). Cells pretreated with TPA expressed an additional kind of channel activity, distinguished by a larger unitary amplitude at any given potential, and a slope conductance of ≥20 pS. The large conductance channel was also induced when protein kinase C was stimulated by pretreatment o

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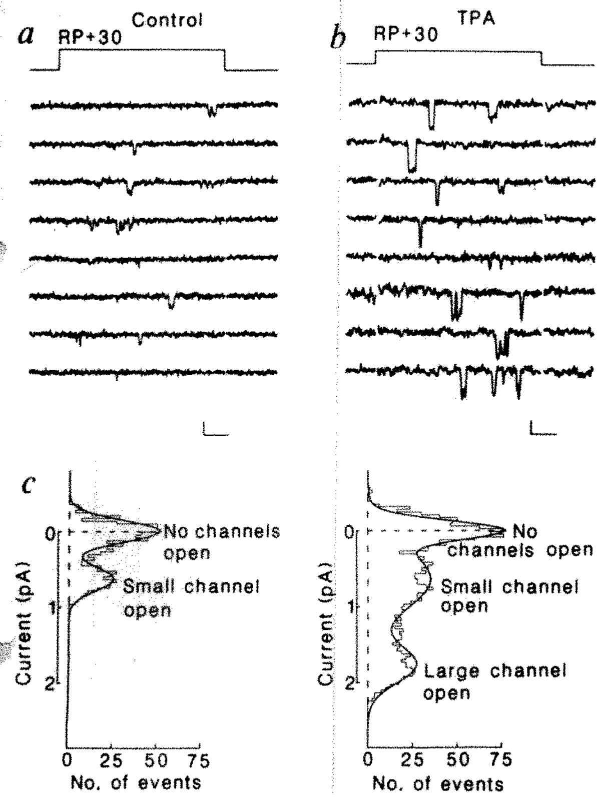

Fig. 1 Examples of two types of inward channel activity in cell-attached patches on bag cell neurons in primary culture. Downward deflections correspond to inward current. Leak and capacitative currents have been subtracted. Scale bars (vertical) 1 pA, (horizontal) 20 ms in *a*, 10 ms in *b*. *a*, A single class of inward current events observed during depolarizations from ($RP-20$) to ($RP+30$) mV, where *RP*, the resting potential was estimated as ~ -48 mV (Fig. 2 legend). *b*, Inward current events of two distinct sizes in another cell pretreated with 10 nM TPA. Pulses from *RP* to ($RP+30$) mV. Estimated $RP \sim -61$ mV. *c*, Histograms of current amplitude for the experiments shown in *a* (left) and *b* (right). The left histogram is fit with the sum of two gaussian functions about 0 and 0.72 pA; the right with the sum of three gaussian functions about 0, 0.69 and 1.74 pA.

Methods. *Aplysia* bag cell neurons were enzymatically and mechanically isolated, plated onto plastic tissue culture dishes, and maintained in primary culture for 1-5 days[26]. Cells were incubated in modified Eagle's medium (MEM) containing 460 mM NaCl, 10.4 mM KCl, 27.5 mM $MgCl_2$, 27.5 mM $MgSO_4$, 11 mM $CaCl_2$, 5.5 mM glucose, and 15 mM HEPES-NaOH (*p*H 7.8), supplemented with essential and nonessential amino acids, vitamins, penicillin and streptomycin (Grand Island Biological). During recordings, cells were bathed in medium containing chloride in place of sulphate and, in some cases, with supplements omitted. Single channel recordings were made in the cell-attached configuration[16], with Sylgard-coated patch pipettes containing 185 mM $BaCl_2$, 15 mM HEPES-TEA-OH (*p*H 7.8). TEA-Cl (260 mM), CsCl (10 mM), 4-aminopyridine (20 mM) were also included in the pipette solution to help block outward channels; preliminary experiments showed that outward channel activity was often detectable when the pipette contained isotonic $BaCl_2$ only. Phorbol ester stock solutions were 1-5 mM in DMSO, and were stored at −5 °C. This and all subsequent current records were filtered with a corner frequency of 1 kHz and sampled at 5 kHz, except the record in Fig. 1*b* which was sampled at 10 kHz (note change in timescale).

neurons with the synthetic diacylglycerol DOG (Figs 2 and 3; Table 1). Some patches contained both a large and small channel. An example can be seen in Fig. 1*b*, where openings of two different sizes can be seen (1st and 3rd traces), and the histogram of current amplitudes has two non-zero peaks (Fig. 1*c*).

Further evidence for the existence of two distinct channel sizes is shown in Fig. 2. Figure 2*a* shows results from 10 patches in which the single-channel current was accurately measured at three or more different potentials. The single channel conductances determined by linear regression clustered about two values, 12 and 24 pS, and none of the 10 channels had conductance values between 14 and 22 pS. The large channels were seen only in cells pretreated with activators of protein kinase C. As Fig. 2*a* shows, the conductance of the small channel is the same in control and TPA-treated cells. The slope conductance of the large channel is the same in TPA-pretreated and DOG-pretreated cells. For both small and large channels, the current-voltage relation extrapolates to a positive reversal potential. The true reversal potential is likely to be even more positive, if these channels show the marked rectification characteristic of calcium channels studied in other systems[17-20].

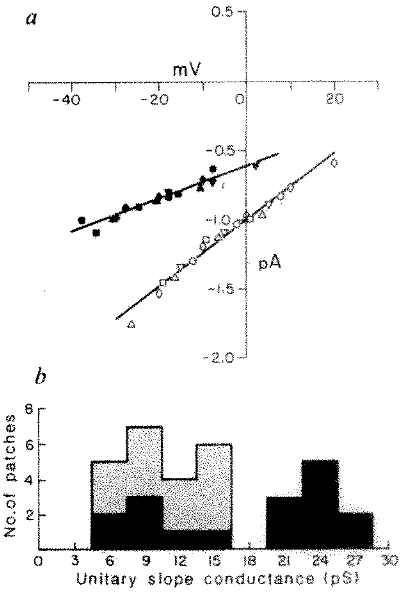

Fig. 2 *a*, Unitary current amplitudes as a function of potential for control channels and channels induced by stimulation of protein kinase C. In each of 10 different patches, averaged measurements of the magnitude of single channel currents were made at 3 or more potentials. The conductance of each channel (γ) was determined by linear regression. Solid symbols, open channel currents of 'small' channels (defined as those with $\gamma < 16$ pS; average value, 11.6; range 8.7–13.8), measured in 5 different patches (◆, ▼, control cells; ●, TPA-pretreated cell; ▲, ■, recordings with pipettes containing TPA). The points have been fitted with a straight line with a slope conductance of 11.8 pS and a voltage intercept of 52 mV. Open symbols, open channel currents of 'large' channels (defined as those with $\gamma > 20$ pS; average value, 23.8; range 22.7–27.8), measured in 5 different patches (□, ◇, △, cells pretreated with TPA; ▽, ○, cells pretreated with DOG). Points are fitted with a straight line with a slope of 24.0 pS and voltage intercept of 42 mV. Measurements from one large and one small channel (◆, ◇) are from cells depolarized to 0 mV by an extracellular medium containing: potassium aspartate (535 mM), potassium EGTA (15 mM) and HEPES-KOH (15 mM, *p*H 7.8). These two channels were used to estimate resting potentials in other patch clamp recordings. Resting potentials estimated in this way ranged from −20 to −60 mV; a very wide range of resting potentials is also observed with microelectrode recordings. *b*, Distribution of slope conductances for channels included in Table 1. Stippled area, channels from control cells and from recordings made with TPA inside the pipette; solid area, channels from cells pretreated with TPA (10–200 nM) or DOG (1.5 μg ml^{-1}). Because *b* includes estimates of single channel conductances based on measurements at only two potentials it has greater scatter in conductance values than *a*. No data from patches containing more than one size of channel are included.

The results illustrated in Figs 1 and 2*a* are representative of a large number of cell-attached patches (Table 1, Fig. 2*b*). Large

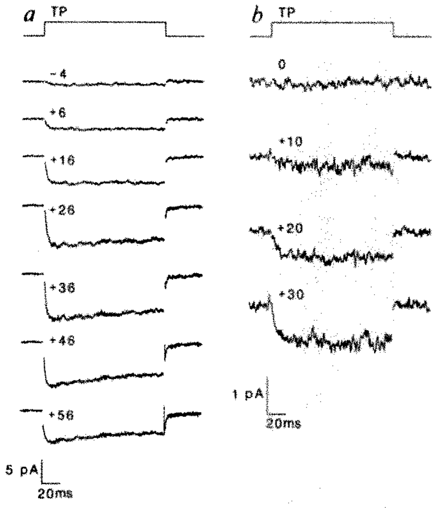

Fig. 3 Voltage dependence and time dependence of currents associated with activity of small and large conductance channels. *a*, Mean currents obtained from a patch containing many small conductance channels and no detectable activity of large conductance channels. Estimated holding potential −54 mV, test potentials (TP) as indicated. No drug pretreatment. At +30 mV, the ratio of peak current to single channel current is 25. This represents a lower bound on the number of channels in the patch, as the probability of a channel being open is much less than 1. *b*, Mean currents obtained from a patch containing at least 5 large conductance channels and no detectable activity of small conductance channels. Holding potential −20 mV, test potentials as indicated. Pretreatment with 1.5 μg ml^{-1} DOG.

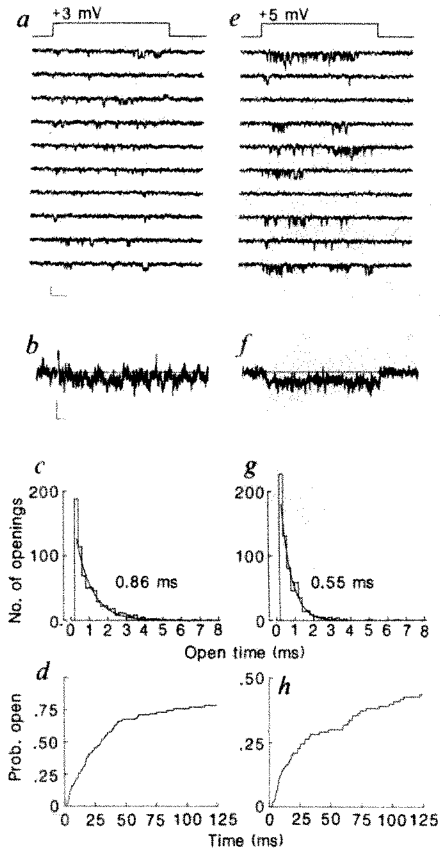

Fig. 4 Kinetics of small and large conductance channel openings studied in cell-attached patch recordings. Recording conditions as in Fig. 1. *a–d*, Analysis of small conductance channel openings associated with patch depolarization from −47 to +3 mV in a two-channel patch. No drug pretreatment. *e–h*, Activity of large conductance channel produced by patch depolarization from −95 to +5 mV in a two-channel patch. Pretreatment with 10 nM TPA. *a*, *e*, Sets of 10 consecutive records. Scale bars 1 pA and 20 ms (*a*, *b*, *e*, *f*). *b*, *f*, Associated mean current records, obtained by averaging more than 100 current traces. *c*, *g*, Distributions of open times; smooth curve indicates least-squares single exponential fit. *d*, *h*, Cumulative distribution of latency-to-first-opening (corrected for the presence of two channels). Similar values were obtained in analysis of one other small channel patch and three other large channel patches. Openings were defined as events in which the current exceeded half of the unit current size for at least 2 sample points. Note that vertical scales are different in *d* and *h*.

conductance channels were never seen in 34 control patches (untreated cells, $n = 21$; cells pretreated with the inactive analogue 4-α-phorbol, $n = 13$). They were found in 36% of all patches from cells pretreated with low concentrations of TPA (10–20 nM).

In Fig. 3, the voltage dependence and time dependence of the small and large conductance Ca channels are compared. Figure 3*a* shows signal-averaged current traces from a control patch with at least 25 small conductance channels; Fig. 3*b* shows records from a patch on a DOG-pretreated cell with about 5 large conductance channels but no detectable activity of the other type. Both channel types become active in a steeply voltage-dependent manner over the range of potentials from 0 to +30 mV. In both cases, the average current activates rapidly and shows little inactivation over the course of a 136 ms depolarizing pulse. In all these respects, the behaviour of the two types of channels is consistent with the pattern of modulation found previously in whole-cell recordings where TPA pretreatment enhanced the magnitude of the calcium current without obvious changes in the voltage or time dependence of the current[13].

A detailed analysis of unitary recordings was carried out to compare the open–closed kinetics of the two types of channels. Figure 4 shows results from patches with only the small conductance channel (left) or only the large conductance channel (right). There is no striking difference in the kinetics of the small and large conductance channels. In both cases, the open time distribution is fitted by a single exponential with a time constant <1 ms, and the cumulative distribution of the latency to first opening rises relatively slowly. Openings of the large conductance channels showed a greater tendency to appear grouped in closely spaced bursts (see Fig. 4*e*).

In addition to differences in single channel conductance and bursting kinetics, another distinguishing characteristic may be spatial distribution. In 25% of the patches containing small channels we observed clusters of dozens of channels (Fig. 3*a*); this was never true for the large conductance channels. In hope of finding clear-cut pharmacological differences between the two types of calcium channel, we tested a variety of calcium channel blockers. La^{3+} (5–30 μM) and nifedipine (5–10 μM) blocked calcium currents with similar effectiveness in whole cell recordings from control and TPA-pretreated cells. No clear results were obtained with Cd (2–50 μM) which appeared to be toxic to the cells.

These experiments demonstrate that modulation of Ca entry in bag cell neurons occurs through the recruitment of a covert class of Ca channels that are readily distinguished from the Ca channels that are active in unstimulated cells. Although we cannot completely rule out the possibility that the large channels arise from conversion of small channels, several pieces of evidence argue against this hypothesis. The percentage of patches containing small channels does not decrease in TPA-treated cells (Table 1), and, moreover, the two channels differ not only in single channel conductance and bursting kinetics but also in their spatial distribution.

The mechanism of Ca channel regulation that we have described seems fundamentally different from the pattern of modulation found so far in other systems of voltage-gated ion channels. Previous studies have described modulation of ion current arising from changes in the availability or opening probability of an apparently homogeneous class of channels. This is true for L-type Ca channels in heart muscle cells[8-12] or S-type K channels in *Aplysia* sensory neurons[21], two particularly well-studied cases where channels are modulated through the action of cAMP-dependent protein kinase.

Several pieces of evidence favour the idea that the large conductance Ca channel appears as a result of activation of protein kinase C. (1) In the experiments with TPA, the concentration of the phorbol ester (10 nM) is similar to that needed to activate protein kinase C in homogenates of *Aplysia* nervous system[13,14]. (2) In mammalian brain, protein kinase C is the only known receptor of phorbol esters at such concentrations[22]. (3) No large channels were seen in cells treated with 4-α-phorbol, a phorbol compound that does not activate protein kinase C. (4) Large channels were also induced by pretreatment of bag cell neurons with DOG, a diacylglycerol activator of protein kinase C. The appearance of the large conductance channels cannot be secondary to changes in electrical activity as microelectrode recordings indicate that 10–20 nM TPA has no effect on resting potential and does not initiate spontaneous activity[13].

An intriguing aspect of the induction of the large conductance channel is that the process is somehow prevented by the formation of cell-attached patches (Table 1). Although large channels were found in 63% of the non-blank patches on cells pretreated with TPA (10 nM), they were never found when TPA (100 nM) was added to the bath after formation of the patch (6 patches with no channel, 5 patches with small conductance channel). In addition, no large channels were observed in 8 recordings made with 10 nM TPA in the patch pipette solution. In this vein it is worth noting that the acute effect of TPA is also prevented by intracellular dialysis of bag cell neurons during whole cell recording (although TPA enhancement of Ca action potentials is readily observed with conventional microelectrodes). The recruitment of large conductance Ca channels may depend upon cellular constituents or structural elements of the native cytoplasm that are lost during dialysis or disrupted by formation of cell-attached patches.

The results in this paper provide a possible mechanism for effects of protein kinase C stimulation in other neuronal systems, including increased Ca^{2+} entry, transmitter release, and long-term potentiation of synaptic efficacy[23-25].

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Involvement of dihydropyridine receptors in excitation–contraction coupling in skeletal muscle

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The transduction of action potential to muscle contraction (E–C coupling) is an example of fast communication between plasma membrane events and the release of calcium from an internal store, which in muscle is the sarcoplasmic reticulum (SR)[1]. One theory is that the release channels of the SR are controlled by voltage-sensing molecules or complexes, located in the transverse tubular (T)-membrane, which produce, as membrane voltage varies, 'intramembrane charge movements'[2], but nothing is known about the structure of such sensors. Receptors of the Ca-channel-blocking dihydropyridines present in many tissues[3], are most abundant in T-tubular muscle fractions[4,5] from which they can be isolated as proteins. Fewer than 5% of muscle dihydropyridines are functional Ca channels[6]; there is no known role for the remainder in skeletal muscle physiology. We report here that low concentrations of a dihydropyridine inhibit charge movements and SR calcium release in parallel. The effect has a dependence on membrane voltage analogous to that of specific binding of dihydropyridines[6]. We propose specifically that the molecule that generates charge movement is the dihydropyridine receptor.

Transient increases in myoplasmic $[Ca^{2+}]$ ('Ca transients') associated with pulse depolarization of single fibres of frog semitendinosus, were obtained by changes in light absorbed by a calcium-sensitive dye in the myoplasm[7]. Figure 1*a* illustrates the effect of nifedipine on Ca transients, elicited at a holding potential (h.p.) of −70 mV (left) or −100 mV (right). In this and four other experiments nifedipine caused a large reduction in the Ca transient at h.p. = −70 mV (Fig. 1*a* trace 4) and a much smaller reduction at h.p. = −100 mV (Fig. 1*a* trace 3). That the effect was small at h.p. = −100 mV may explain a recent negative report on effects of nifedipine on contraction[8].

The Ca transients are the result of release of Ca from the SR and removal of calcium by various cell structures and the dye. To separate the effects of nifedipine on release alone, we derived from the Ca transients the 'rate of release'[9] or release flux[10] (Fig. 1*b*), representing the flux of calcium into the myoplasm per unit time and unit myoplasmic water volume. The effects on release flux were much greater, in both relative and absolute terms, at h.p. = −70 mV; in this and two other experiments 500 nM nifedipine caused a reduction of about 70% after 20 min exposure (Fig. 1*b*, record 4) and greater than 80% reduction after 30 min (two fibres, Fig. 3*b*). At h.p. = −100 mV after more than 20 min the effect was a 6% reduction (s.e.m. = 6%, $n = 5$).

The 'release' waveform lumps together all contributions that increase myoplasmic Ca^{2+}, including release from the SR and calcium influx via the T-membrane Ca channel[11]. In principle, the decrease in the release waveform caused by nifedipine could be due to blockage of the T-membrane Ca channel and abolition of calcium influx through it. We estimated the effect of nifedipine on the T-membrane Ca current by subtracting total current in nifedipine from the total membrane current. This difference was negligible compared with the reduction of release flux caused

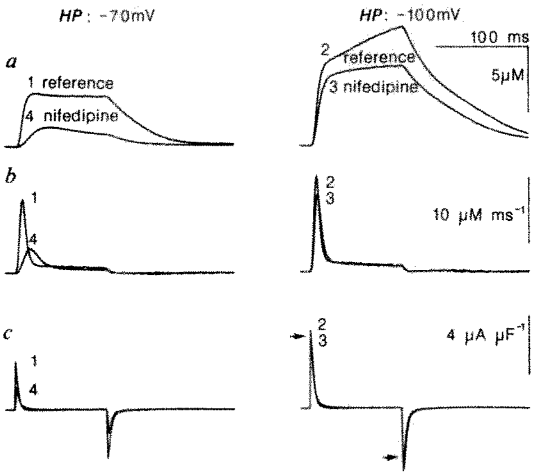

Fig. 1 *a*, Time course of myoplasmic [Ca^{2+}] derived from absorbance changes in the presence of the dye Antipyrylazo III[28,29] in a cut skeletal fibre under voltage clamp. A detailed description of the technique has been published[30]. *b*, Rate of release[9] or release flux[10] records derived from the records in *a*. *c*, Records of asymmetric membrane current ('charge movement'), corresponding to the calcium records in *a* and *b*.

Methods. In *a*, the cut ends of a fibre segment were exposed to an internal solution containing 0.8 mM of Antipyrylazo III (K&K Labs). The central portion of the fibre was voltage clamped and polarized to h.p. = −70 mV (left-hand records) or −100 mV (right-hand records) in a double vaseline gap chamber with a glass bottom. Light from a tungsten-halogen 100 W bulb was filtered to eliminate wavelengths <650 mM and focused on the fibre. The transmitted light was collected with an immersion objective and its intensity recorded at 700 nm and 850 nm. The intensity record at 700 nm was corrected for intrinsic changes in absorbance (recorded at 850 nm) and processed to derive time course of [Ca^{2+}][30]. The applied pulse went in all cases shown from −70 mV to 0 mV (100 ms). Records 1 and 2 were obtained in reference external solution, 3 and 4 after 20 min in the same solution plus 500 nM high-purity nifedipine (gift from Pfizer Laboratories). The records were obtained in the sequence 1,2,3,4: other experiments with different sequences gave similar results. In *c*, records were obtained as differences between the total membrane current during the test pulse and a control current recorded previously with the fibre held at h.p. = 0 mV during a pulse to +70 mV[12]. A sloping baseline was fitted to the last 50 ms of the ON and OFF portions of the difference and subtracted. Arrows, peaks of record 3 at ON and OFF. Fibre diameter, 55 μm; length under clamp, 540 μm. Calibration bar, 30 nA or 32 μA cm^{-2} surface area. Temperature 11 °C. Composition of solutions: tetraethylammonium-sulphate, 75 mM; Cs_2SO_4, 5 mM; caesium Tris-maleate buffer, 5 mM; $CaSO_4$, 10 mM; tetradotoxin (TTX), 10^{-7} g ml^{-1}; 3-4 diaminopyridine, 1 mM. Internal solution (in end compartments of the double vaseline gap): caesium glutamate, 108; $MgCl_2$ 5.5; sodium Tris-maleate, 4.5; caesium Tris-maleate, 13.2; EGTA, 0.1; $CaCl_2$ 0.0082; Na_2 ATP, 5; glucose, 5; Antipyrylazo III, 0.8; *p*H 7.0. Data acquisition and pulse generation performed with a laboratory computer (LSI-11/23 PLUS, DEC) with A/D and D/A conversion boards (DT 3382 and D7 3371, Data Translation)[12].

by the drug, indicating that nifedipine actually reduces Ca release from the SR.

The effect is probably mediated by a direct action of the drug on the voltage sensor of E–C coupling. Figure 1*c* shows the charge movement records that correspond to the calcium records in *a* and *b*; there is a substantial effect of nifedipine at h.p. = −70 mV and a small effect at h.p. = −100 mV. A more detailed exploration of effects on charge movement at h.p. = −70 mV (Fig. 2) showed that charge moved during a depolarizing pulse to 0 mV was much smaller after 10 min in 100 nM nifedipine (records 3 and 4, Fig. 2*a*).

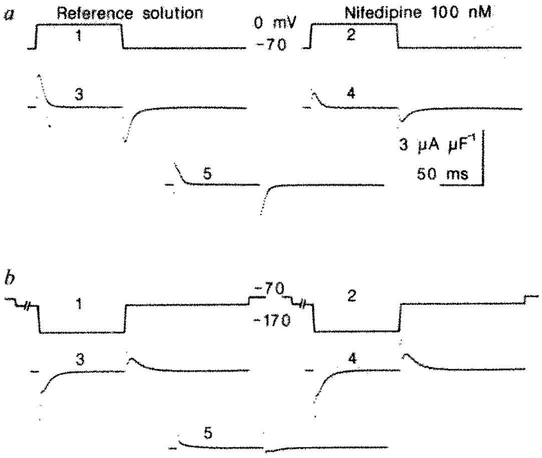

Fig. 2 Effect of nifedipine on intramembrane charge movement. Left, records in reference solution; right, records in 100 nM nifedipine. h.p. = −70 mV. *a*, Recorded membrane voltage (records 1 and 2) and charge movements (records 3 and 4). Record 5, total current during the test pulse in reference solution minus total current during the test pulse in nifedipine (record 3 minus record 4). *b*, Charge movement records during test pulses from −100 mV (prepulse level) to −170 mV. The control current was the same as in *a*. Record 5, total current during the test pulse in reference solution minus total current in nifedipine (record 3 minus record 4). Same solutions as in Fig. 1. Temperature, 7 °C. Fibre diameter, 75 μm. Calibration bar, 33 nA or 26 μA per cm^2 surface area.

The records of charge movement are obtained by subtracting a ('control') linear capacitive current from the total current recorded during the test pulse. Conventionally, control currents are determined with negative pulses from −100 mV. We recently demonstrated[12] that records obtained with positive pulses from a h.p. = 0 mV better satisfy the criteria of linearity required for control pulses. Using these controls it is clear that charge moves in the range of voltages negative to −100 mV (Fig. 2*b*). Charge movement in reference solution (Fig. 2*b* record 3) or nifedipine (record 4) for a pulse from −100 mV to −170 mV was measured. Surprisingly, in 8 out of 9 fibres studied the charge movement increased in the presence of nifedipine. The average increase in charge moved for a pulse from −100 mV to −170 mV was 3.7 nC μF^{-1} (0.87 s.e.m.). Thus, nifedipine not only decreases the charge that moves positive to −70 mV (refs 13, 14) but also increases the charge that moves negative to −100 mV. The effects are similar in this regard to those of prolonged depolarization[12], which include an increase in charge mobile at large negative potentials, probably corresponding to transitions between inactivated (or nifedipine-bound) states of the voltage sensor[12].

Table 1 summarizes data on concentration-dependence. Substantial effects were observed even at 10 nM, provided that the drug was perfused continuously for 20 min or more. Figure 3*a* and *b* represents the time course of onset and recovery of the effect on peak release flux and total charge moved during a pulse to 0 mV. The effects on charge movement and calcium release were essentially cotemporal. The onset was slow and, especially at higher concentrations, the effects still increased after 30 min of drug exposure.

The experiment in Fig. 3*b* demonstrates photoinactivation of the drug[15]. After 15 min nifedipine treatment of the specimen, filters that normally eliminate incident light of short wavelength were removed at times (arrows) for brief intervals. This caused rapid but incomplete recovery of both release and charge. The magnitude of the recovery and subsequent relapse was comparable for both variables.

In Fig. 3*c*, charge movement elicited with pulses positive to h.p. = −70 mV is plotted against peak release in several fibres in reference solution and exposed to different nifedipine con-

Table 1 Effects of three concentrations of nifedipine on charge movement and peak release flux

[Nifedipine] (nM)	10	100	500	500
h.p. (mV)	−70	−70	−70	−100
n (fibres)	3	4	2	5
Reduction in charge movement (%)	36	62	67	29
	(33–38)	(33)	(65–69)	(7.0)
Reduction in peak release (%)	37	67	84	6
	(14–59)	(8.7)	(79–89)	(6.0)

Effects calculated from an average value before nifedipine and the value in nifedipine at the longest exposure time available (between 18 min and 32 min). Average reductions are followed by the s.e.m. (or the range, whenever the sample number is ⩽3) in parentheses. Charge movement figures are averages of ON and OFF. All changes were significant ($P < 0.05$) except that of peak release at h.p. = −100 mV. The reductions of the ON and OFF charges at h.p. = −100 mV (22 and 37% respectively) were significantly different. Temperature, 7–11 °C. Test pulse from −70 to 0 mV.

centrations. The regression line is derived from the points in reference solution. This line describes well the results in nifedipine and passes very close to the centre of mass of the points in nifedipine. Thus, the effect of nifedipine on Ca release seems well explained by its effect on charge movement. However, the regression line does not go through the origin, suggesting that a minor fraction of the charge is not directly involved in signalling release.

Two main results have been reported here: (1) nifedipine inhibits Ca release from the SR, probably by an effect on intramembrane charge movement; (2) the effect is greater at h.p. = −70 mV than at h.p. = −100 mV.

The first result indicates that nifedipine binding molecules are involved in E–C coupling. The second result suggests that these molecules are the high affinity dihydropyridines (DPRs), as binding to DPRs depends on h.p. in a similar way[6]. DPRs have been identified in various membrane preparations[3] but in T-tubular fractions, which constitute the richest source[16], their density is 20–50 times that of membrane Ca channels[6]. In addition to voltage dependence of binding, several of their properties are consistent with the hypothesis that DPRs are the voltage sensors of E–C coupling or closely interact with them in a protein complex. (1) Their density is roughly consistent with that of intramembrane charge movement particles. The density of DPRs in frog muscle is ~230 sites μm^{-2} of T-tubular membrane[6]. The density of charge movement sites was estimated at 500–600 sites μm^{-2} of T-tubular plus surface membrane[17]. (2) Other drugs, like D-600[15] and verapamil[16] interact specifically with the DPRs, perhaps at a different binding site on the same molecule. D-600 both inhibits contractility[18] (presumably by preventing Ca release) and prevents charge movement[19] at concentrations consistent with its affinity for DPRs[20]. (3) Mice with muscular dysgenesis[21] lack E–C coupling[22]. These mice have a very reduced density of DPRs[23] and lack slow Ca currents[24].

From the average value of the effects of 10 and 100 nM nifedipine on release flux a dissociation constant of about 32 nM results for a simple one-site reaction. The dependence of the effect on h.p. suggests that the high affinity is a property of conformations of the receptor different from its resting state, as proposed for Ca channels[25,26]. The simple model:

$$R \rightarrow O \rightarrow I \xrightarrow{K_D} IN$$

in which nifedipine (N) cannot bind to the resting or open states of the sensor (R, O) but only to the inactivated state (I), with dissociation constant K_D, predicts the relationship[25]

$$K_{app}(\text{h.p.}) = K_D/[1 - h_\infty(\text{h.p.})]$$

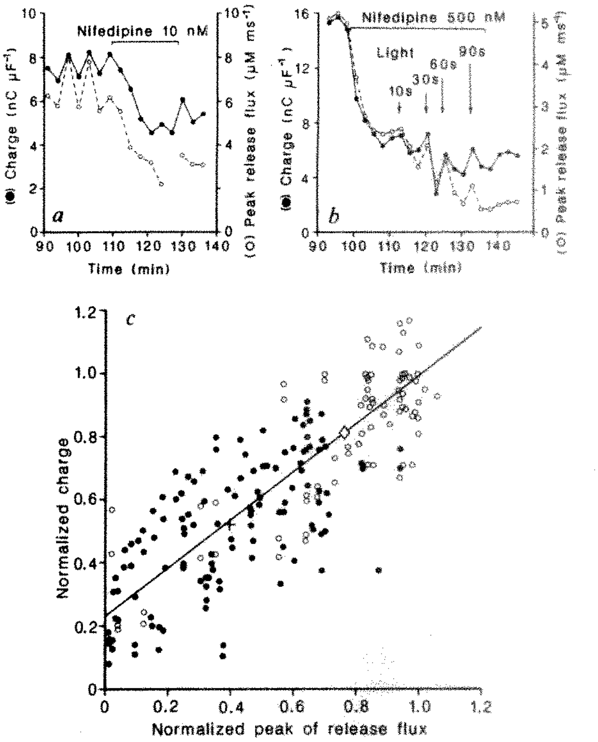

Fig. 3 *a*, *b*, Maximum value of release flux (○) and area under the charge movement transient, average of ON and OFF (●), for pulses from h.p. (−70 mV) to 0 mV in two different fibres exposed to 10 nM (*a*) and 500 nM nifedipine (*b*) during the interval marked by the bars. Temperature, 11 °C. In *a* the release value at 128 min was not measured. In *b* the filter that normally blocks short wavelength radiation was removed from the path of the incident light (used to monitor calcium) at the times indicated by arrows for intervals of 10, 30, 60 and 90 seconds. *c*, Charge moved during depolarizing pulses plotted against peak release flux. Data from 10 fibres. Each fibre was first pulsed in reference solution (○) later in a nifedipine-containing solution (●), at concentrations ranging from 100 nM to 10 μM. The depolarizing pulses were applied from a −70 mV holding potential to different test potentials (−40 mV to +30 mV); about the same voltage pulses were applied in both solutions. Peak release flux and charge movement values, both in reference solution and in nifedipine, were divided by a normalization value, calculated for each individual fibre as the average of the values obtained in reference solution with pulses to 0 mV or greater. The regression line calculated for the values in reference solution has slope 0.762 (0.104 s.d.), intercept 0.231 (0.048 s.d.) and correlation coefficient 0.822. The corresponding values in nifedipine (regression line not shown) are slope 0.590 (0.060 s.d.), intercept 0.285 (0.028 s.d.) and correlation coefficient 0.655. Neither slopes nor intercepts are significantly different. Centres of mass (◇) in reference solution and (+) in nifedipine are also shown.

where K_{app} (h.p.) is the apparent value of the dissociation constant at a holding potential at which the steady value of the inactivation parameter is h_∞(h.p.). This model predicts K_{app} (−70 mV) to be four to five times greater than K_D, as the steady inactivation of release at −70 mV is 0.2 to 0.25 (compare records 1 and 2 in Fig. 1*b*). Therefore the estimate of 32 nM obtained for K_{app} in these experiments corresponds to a K_D of ~7 nM; by comparison the dissociation constant of nifedipine binding is 4 nM (rabbit muscle[16]).

Other possible sites of action of nifedipine should be considered; it is possible but unlikely that depletion of the SR and secondary reduction in its ability to release calcium mediate the nifedipine effects; a rapid recovery after tens of seconds of intense illumination (Fig. 3*b*) seems inconsistent with depletion as the main mechanism. That nifedipine has mainly a direct intracellular effect on the Ca-release channel of the SR, beyond the voltage sensor, or merely an effect of non-specific partition into membranes is also unlikely because of the parallel effect on charge movement and the h.p.-dependence of the effects.

We have described here effects of nifedipine consistent with an involvement of the high affinity DPRs in E-C coupling. DPRs are generally thought of as Ca channels. It is possible that they are channel-like proteins, not necessarily conducting, that perform the voltage sensing function and are coupled to the SR release pathway by unknown means, involving perhaps a mediator[27].

Part of these results have been communicated in abstract form[13]. We thank Dr Enrico Stefani for help and encouragement, Dr G. Pizarro for help with experiments, Drs T. DeCoursey, F. Cohen, R. Eisenberg and W. K. Chandler for discussions and Ms. L. Vaughn for careful typing. Supported by NIH grant AM32808.

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Tγ protein is expressed on murine fetal thymocytes as a disulphide-linked heterodimer

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During the search for genes coding for the mouse α and β subunits of the antigen-specific receptor of mouse T cells we encountered a third gene, subsequently designated γ[1]. This gene has many properties in common with the α and β genes[1,2]; somatic assembly from gene segments that resemble the gene segments for immunoglobulin variable (V), joining (J) and constant (C) regions; rearrangement and expression in T cells and not in B cells; low but distinct sequence homology to immunoglobulin *V*, *J* and *C* regions; other sequences that are reminiscent of the transmembrane and intracytoplasmic regions of integral membrane proteins; and a cysteine residue at the position expected for a disulphide bond linking two subunits of a dimeric membrane protein. Despite these similarities the γ gene also shows some interesting unique features. These include a relatively limited repertoire of the germline gene segments[2-5], more pronounced expression at the RNA level in immature T cells such as fetal thymocytes[6-8] and an apparent absence of in-frame RNA in some functional, αβ heterodimer-bearing T cells or cultured T clones and hybridomas[9,10]. To understand the function of the putative γ protein it is essential to define the cell population that expresses this protein. To this end we produced a fusion protein composed of *Escherichia coli* β-galactosidase and the γ-chain (hereafter referred to a β-gal-γ) using the phage expression vector λgt11 (ref. 11) and raised rabbit antisera against the γ determinants. Using the purified anti-γ antibody we detected a polypeptide chain of relative molecular mass 35,000 (M_r 35K) on the surface of 16-day old fetal thymocytes. The γ-chain is linked by a disulphide bridge to another component of 45K. No such heterodimer was detected on the surface of a cytotoxic T lymphocyte (CTL) clone 2C[12] from which an in-phase γ cDNA clone was originally isolated.

We inserted various parts of the γ cDNA clone pHDS4/203 which contains an in-phase $V_2J_2C_2$ sequence (see ref. 4 and 9 for the nomenclature of mouse γ gene segments) into the *Eco*RI cloning site of the phage expression vector λgt11 and determined the proportion of the β-gal-γ fusion proteins synthesized in the various lysogens by SDS-polyacrylamide gel electrophoresis (PAGE) analysis (data not shown). We then chose the phage construct λgt11-γ1 depicted in Fig. 1*a* because it gave the highest yield of the fusion protein (~4% of the total protein upon induction by isopropyl-thio-β-D-galactoside (IPTG). This γ insert contains almost all the $V_\gamma J_\gamma$ region and part of the *C* region and should code for 15K of the γ polypeptide chain. Figure 1*b* shows Coomassie Blue-stained gels of the lysates prepared from the λgt11-γ1 lysogen without and with induction by IPTG (lanes 2 and 3). The lysate of the λgt11 lysogen with induction is also shown (lane 1). It is apparent from these gel patterns that the 131K band observed in lane 3 is the expected β-gal-γ fusion protein.

The fusion protein was purified by centrifugation followed by preparative SDS-PAGE. As shown in Fig. 1*b* lanes 4-7, the fusion protein can be thus purified close to homogeneity. After removing most of the bound SDS by extensive dialysis, the purified fusion protein mixed with complete Freund's adjuvant was injected subcutaneously into rabbits. Anti-γ-chain antibodies were purified from the sera of hyperimmunized rabbits by first removing anti-β-galactosidase antibody on a β-galactosidase immunoadsorbent and then applying the flow-through to a second immuno-adsorbent composed of the β-gal-γ fusion protein conjugated with Sepharose beads.

To demonstrate that the material eluted from the fusion protein immunoabsorbent indeed contains anti-γ activity we prepared γ protein or fragments of it by two different methods. First, 1 μg of $V_2J_2C_2$ γ RNA was prepared *in vitro* using the SP6 transcription system (Fig. 2*a*)[13,14]. The RNA was then translated *in vitro* into the γ protein using a rabbit reticulocyte lysate. Figure 2*b* lane 1 shows a SDS-PAGE display of the ^{35}S-labelled γ-chain that has been admixed before electrophoresis with the rabbit β globin similarly synthesized *in vitro*. That the purified putative anti-γ antibodies but not the anti β-galactosidase antibody indeed contain anti-γ antibody is demonstrated by the specific immunoprecipitation of the *in vitro* synthesized γ-chain by the former (Fig. 2*b*, lanes 2-5).

Alternatively we prepared a fusion protein composed of mouse immunoglobulin κ chain and the γ-chain constant region by transfecting a myeloma cell J558L with an appropriately constructed chimaeric plasmid. Thus the major C_γ exon and its flanking sequences were inserted in the major intron of a κ light chain gene (Fig. 3*a*). The myeloma cells that have been stably transfected with the plasmid secrete, albeit in small amounts, the $V_\kappa C_\gamma C_\kappa$ three-domain polypeptide chain into the medium

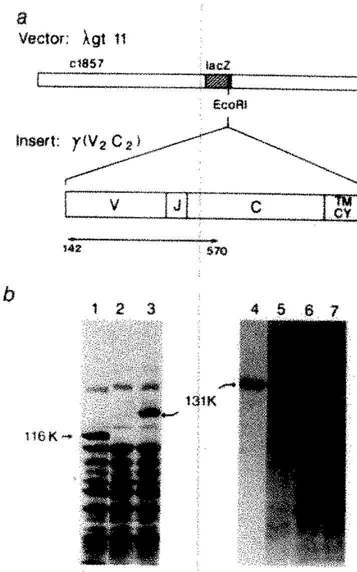

Fig. 1 Synthesis and purification of β-galactosidase-γ fusion protein. *a*, Construction of the chimaeric phage λgt11-γ1. A 429-base pair (bp) DNA fragment coding for the entire *VJ* region and part of the *C* region of the γ cDNA clone pHDS4/203 (ref. 1) was inserted into the *Eco*RI site of the phage λ expression vector λgt11 (ref. 11). *b*, Purification of the fusion protein. Aliquots of the lysates prepared from λgt11 or λgt11γ1 lysogens or fractions thereof were analysed by SDS-PAGE. The gel was stained with Coomassie Blue. Lane 1, λgt11 lysogens induced by IPTG; lane 2, λgt11-γ1 lysogen uninduced; lanes 3 and 7, induced λgt11-γ1 lysogen; lanes 5 and 6, pellet and supernatant fractions (respectively) after a low speed centrifugation of the lysate of the induced λgt11-γ1 lysogens; lane 4, fusion protein eluted from a preparative PAGE run.

Methods. $V_2J_2C_2$ γ cDNA clone pHDS4/203 was digested with *Pvu*II and *Bst*XI and the 429-bp fragment (nucleotide positions 142-570; see ref. 3 for the numbering system used) was purified, blunt-ended by T_4 polymerase, and ligated into the unique *Eco*RI site of λgt11 DNA using *Eco*RI linkers. The recombinant DNA was packaged *in vitro* using the λ phage packaging mix (Promega) and the packaged phages were plated on *E. coli* Y1088 (sup F^+). The phages containing the γ DNA sequence in the appropriate orientation were identified by plaque hybridization followed by restriction enzyme analysis of the purified phage DNA. The lysogens prepared with these phages as well as the vector phage λgt11 were grown and the β-galactosidase or the β-gal-γ fusion gene was induced with IPTG by the standard procedure[11]. The bacterial cells were collected in a lysis buffer (0.2 M Tris-HCl *p*H 7.6, 0.2 M NaCl, 1 mM EDTA, 5% glycerol, 1 mM dithiothreitol (DTT), 1 mM PMSF (phenylmethylsulphonyl fluoride) and 100 units ml^{-1} Aprotinin and disrupted by freezing and thawing, then sonication. The lysate was centrifuged at 20,000*g* for 40 min at 4 °C. The pellet was washed once with the above buffer. For the preparative SDS-PAGE the washed pellet was suspended in the lysis buffer. The proteins were dissolved by addition of one volume of 2-fold concentrated SDS-PAGE sample buffer (160 mM Tris-HCl *p*H 6.8, 4% SDS, 20% glycerol, 4 mM EDTA, 10% β-mercaptoethanol) and electrophoresed in 6% cylindrical acrylamide gels of 15 mm diameter. The 131K protein was electroeluted in the electrophoresis buffer (25 mM Tris, 192 mM glycine, 0.1% SDS) and dialysed against a buffer containing 50 mM Tris-HCl *p*H 7.5, 2.5 mM octylglucoside, 0.1 mM PMSF, followed by the same buffer without octylglucoside.

ong with the endogenous λ light chain (Fig. 3*b*, lane 1). The sults shown in the rest of Fig. 3*b* show that the fusion protein specifically precipitated with the serum of the rabbit hyperimunized with the β-gal-γ fusion protein. These results not only nfirm the rabbit serum contains anti-γ-antibody but also monstrate that at least some of this antibody is directed against e C_γ region.

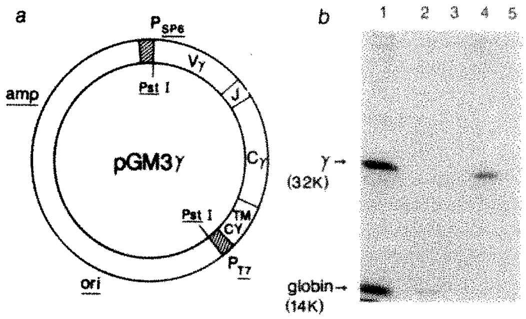

Fig. 2 Immunoprecipitation of the *in vitro*-synthesized γ polypeptide chain by the putative anti-γ antibody. *a*, Construction of a plasmic, pGM3γ, for the *in vitro* transcription by SP6 RNA polymerase. The γ cDNA pHDS4/203 was subjected to limited digestion with *Pst*I and the 1372-bp fragment (nucleotide positions 43-1414 (ref. 3)) containing the entire translated region and some flanking untranslated regions of the γ cDNA was ligated to the *Pst*I site of pGEM3 (Promega). This vector carried the transcription promotor for SP6 RNA polymerase just upstream of the *Pst*I site. *b*, A SDS-PAGE autoradiogram of the immunoprecipitate of the *in vitro*-synthesized γ polypeptide chain. The ^{35}S-labelled γ protein synthesized *in vitro* was mixed with the similarly synthesized rabbit β globin (lane 1) and precipitated with a rat anti-rabbit-globin (lane 2), a normal rat serum (lane 3), the anti-γ fraction (lane 4) and anti-β-galactosidase fraction (lane 5) of the serum of the rabbit hyperimmunized with the β-gal-γ fusion protein.

Methods. pGM38 DNA was linearized with *Bam*HI transcribed by SP6 RNA polymerase (Promega) as described[13,14]. The template DNA was digested with RNase-free DNase and the RNA was extracted by a phenol/chloroform mix (1:1), precipitated by 2.5 volumes of ethanol, and extensively washed with 80% ethanol. The purified γ RNA was translated into ^{35}S-labelled protein using the lysate of rabbit reticulocytes (Promega) according to the procedures recommended by the supplier. In order to raise the anti-β-gal-γ fusion protein, rabbits were immunized subcutaneously with the 1 mg purified fusion protein with complete Freund's adjuvant. After three weeks the rabbits were boosted with 1 mg of the fusion protein with incomplete Freund's adjuvant and bled 7 d later. The immunoglobulin was precipitated from the antisera with one volume of saturated ammonium sulphate, dialysed extensively against a phosphate-buffered saline (PBS) and passed through a column composed of β-galactosidase (Sigma)-conjugated Sepharose 4B. The material adsorbed was eluted from the column with 0.2 M glycine-HCl buffer (*p*H 2.6) (anti-β-galactosidase fraction). The effluent was passed through a second affinity column containing the purified fusion protein and the material adsorbed (anti-γ fraction) was similarly eluted. Immunoprecipitation was carried out by standard procedures using protein A-Sepharose.

We next investigated whether a bona fide γ protein could be detected on murine cells of the T-lineage. We chose fetal thymocytes and a CTL clone 2C because these cells were previously shown to contain a relatively high level of γ RNA[1,6-8]. Furthermore the 2C γ RNA was shown to be in-phase[1]. Cells were surface-labelled with Na^{125}I and lactoperoxidase and lysed in 1% NP40. The lysates were subjected to immunoprecipitation with the affinity-purified anti-γ antibody. As a negative control, the same lysates were immunoprecipitated with the anti-β-galactosidase antibody also affinity-purified from the same hyperimmune rabbit serum from which the anti-γ antibody was purified. The immunoprecipitate was analysed by SDS-PAGE under non-reducing conditions in one dimension and then under reducing conditions in the second. Under these conditions those cell-surface proteins containing interchain disulphide linkages migrate differently in the first and second dimensions and are usually displayed in the region below the diagonal line[15]. As shown in Fig. 4*a*, two off-diagonal spots of 45K and 35K are

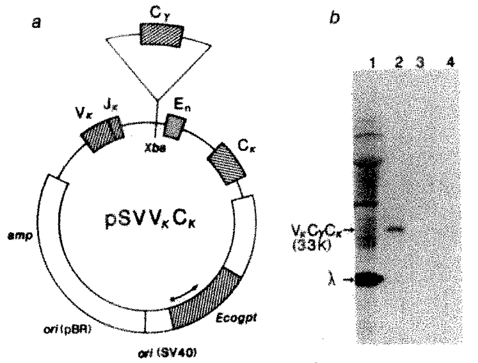

Fig. 3 Immunoprecipitation by the rabbit anti-γ antiserum of the $V_\kappa C_\gamma C_\kappa$ polypeptide chain secreted from a myeloma transfectant. *a*, Construction of the recombinant plasmid pSV $V_\kappa C_\kappa$ containing the C_γ exon within the major intron of a rearranged κ gene. The *Eco*RI-*Bam*HI DNA fragment containing the entire rearranged κ gene was cloned from a mouse myeloma LPC-1 and inserted to a plasmid pSV2gpt[26]. The *Hin*dIII DNA fragment containing major $C_{\gamma 2}$ exon[2] and its flanking sequences was then inserted at the unique *Xba*I site between the $V_\kappa J_\kappa$ exon and the enhancer (En). *b*, An SDS-PAGE autoradiogram of the immunoprecipitate of the $V_\kappa C_\gamma C_\kappa$ protein. ^{35}S-methionine-labelled supernatant of the culture of myeloma J558L stably transfected with pSV $V_\kappa C_\gamma C_\kappa$ (lane 1) was immunoprecipitated by goat anti-mouse κ light chains (lane 2), anti-β-galactosidase fraction (lane 3) or the anti-γ fraction of the rabbit serum obtained after hyperimmunization with the β-gal-γ fusion protein. J558L secretes a λ light chain in large amounts and some of it is carried over non-specifically in the immunoprecipitate.

Methods. A mouse myeloma J558L was transfected with pSV $V_\kappa C_\gamma C_\kappa$ by the protoplast fusion method as described previously[27]. The culture of stable transfectant D8.2 was fed with 0.4 mCi per ml ^{35}S-methionine (Amersham) for 5 h. The protein in the culture supernatant was denatured and partially renatured as described[20] and was subjected to immunoprecipitation and SDS-PAGE.

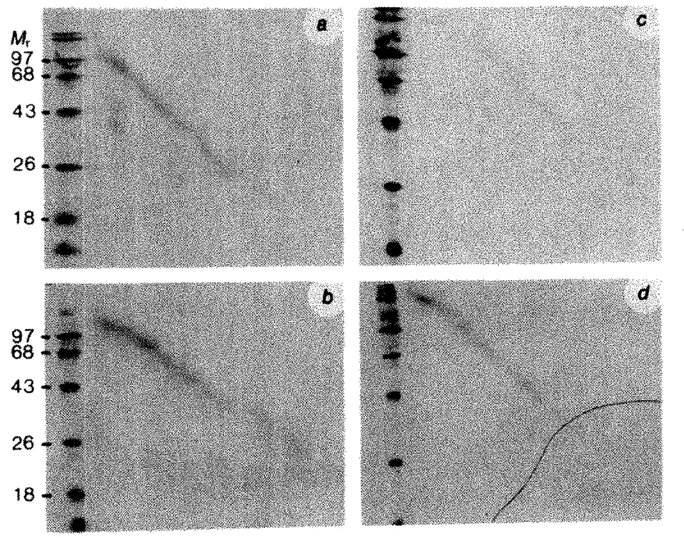

Fig. 4 Two-dimensional PAGE analysis of immunoprecipitated γ ^{125}I-labelled proteins from fetal thymocytes and CTL clone 2C. *a*, *b*, Lysates of fetal thymocytes from day 16 embryos immunoprecipitated by the anti-γ and anti-β-galactosidase fractions, respectively. *c*, *d*, Lysates of CTL clone 2C immunoprecipitated by anti-γ and anti-β-galactosidase, respectively. PAGE was performed under nonreducing conditions from left to right and reducing conditions from top to bottom.

Methods. Fetal thymi were dissected from the embryos of the day 16 pregnant mothers. The thymocytes were suspended in PBS and surface-labelled with ^{125}I using lactoperoxidase. The cells were washed three times with PBS, suspended in cold NP40 buffer (10 mM Tris-HCl *p*H 7.5, 0.1 M NaCl, 1 mM PMSF and 1% Nonidet P40) and disrupted by vigorous shaking. The lysates were subjected to immunoprecipitation and the immunoprecipitates were electrophoresed under nonreducing conditions through 10% acrylamide gels formed in a glass tube of internal diameter 1.5 mm. The gel was then removed from the glass tube, soaked in a buffer containing 5% β-mercaptoethanol for 30 min at room temperature, and placed on top of 12% slab gel for electrophoresis in the second dimension. The CTL clone 2C was analysed similarly.

present in the autoradiogram obtained with the lysate prepared from day 16 fetal thymocytes and the anti-γ antibody. No such spots were detectable when the anti β-galactosidase antibody was used (Fig. 4*b*).

The results of the analysis of the CTL clone 2C are shown in Fig. 4*d*. It seems that neither the 45K nor the 35K 'off-diagonal' component is present on the surface of this CTL clone.

The M_r of the $V_2J_2C_2$ γ-chain predicted by the nucleotide sequence is 33K and the chain should have no *N*-linked carbohydrate. Thus the measured molecular mass, 35K, of the smaller of the two 'off-diagonal' components observed in the fetal thymocytes is in good agreement with the predicted value. Preliminary experiments in which lysate was treated with dithiothreitol, immunoprecipitated and analysed by one-dimensional SDS-PAGE show a specific 35K component in the precipitates obtained with the anti-γ antibody which is competed out when excess purified β-gal-γ fusion protein is added to the lysate before immunoprecipitation (data not shown). Thus it seems that the thymocyte population derived from 16-day-old mouse fetuses contains cells that bear on the surface a γ-chain linked by one or more disulphide bonds to a 45K component.

As the anti-γ antibody was prepared by using as the immunogen the polypeptide encoded by a part of $V_2J_2C_2$ cDNA (see Fig. 1*a*), we tacitly assumed that the γ chain represented by the 35K component is of the $V_2J_2C_2$ type. But three more *V* gene segments, V_4, V_5 and V_6, are known to be expressed at least at the RNA level in fetal thymocytes in combination with another *JC* pair, J_1C_1[4,10,11]. Furthermore another γ gene, $V_1J_4C_4$, was recently reported[17]. As J_1C_1 and V_2 are highly homologous to J_2C_2 (94% homology at the protein level) and V_1 (87% homology), respectively, it is possible that the anti-$V_2J_2C_2$ antibodies used in this study crossreact with the γ-chain encoded by any of these four additional types of γ genes. Thus at least some of the 35K material shown in Fig. 4 may be non-$V_2J_2C_2$ γ chain.

The chemical nature of the 45K component is less clear. We cannot rule out the possibility that the 'off-diagonal' 45 K component observed in Fig. 4*a* carries a γ epitope. If this component is a γ-chain, its relative molecular mass seems to rule out the $V_2J_2C_2$ type. On the other hand, because any one of the other four known types of γ-chains may be *N*-glycosylated, its molecular mass could be as large as 45K. Another possible candidate for the 45K component is the β-chain; the β gene is known to be rearranged and activated for transcription by day 16 of fetal thymocytes[6-8]. A model of intrathymic development of T cells including thymocytes bearing a βγ heterodimer has been presented[6,18,19], but no direct evidence for such cells has been obtained to date.

Yet another possible candidate for the 45K component is a product of a new gene referred to as δ. The existence of such a polypeptide is supported by recent work on human systems. Brenner *et al.* reported the identification of a putative second T-cell receptor present on the surface of a subpopulation of peripheral blood lymphocytes (PBL) from immunodeficient patients[20]. This protein is associated with the invariant protein CD3 (T3), and is composed of a 55K component reactive with anti-γ-peptide antibodies and a 40K polypeptide that does not react with these antibodies. Similarly Bank *et al.* reported a clone of normal immature $CD4^-8^-$ ($T4^-T8^-$) human thymocytes which bear CD3-associated dimer composed of anti-γ peptide-reactive 44K component and anti-γ peptide non-reactive 62K component[21]. These authors propose that the component that does not react with the anti-γ peptide is a product of a new gene, δ, although its molecular mass is quite different (40K as against 62K) between the two publications.

The function of the cells bearing the putative $\gamma\delta$ heterodimer remains unknown. It has previously been reported that some CTL clones and many T helper (T_H) clones or hybridomas either lack γ RNA completely or contain only an out-of-phase γ RNA[5,9,10,22]. These data led some to conclude that the protein encoded by the reported γ genes is not an obligatory requirement for the function of these T cells. The present study shows further that even in 2C, a CTL clone in which the γ RNA is present and in-phase, the membrane expression of the γ polypeptide chain is absent and therefore not a prerequisite of the antigen-specific function of the T cell.

One possible view of the putative $\gamma\delta$ heterodimer is that it is 'isotypic' to the conventional $\alpha\beta$ TCR, in a relationship analogous to the κ and λ light chains in immunoglobulins. Indeed the structural resemblance among α, β and γ[23] is reminiscent of the similarity between κ and λ. Furthermore the $\gamma\delta$-bearing cells seem to have cytotoxic[21] or IL-2 producing capacity (D. Pardoll and A. M. Kruisbeek, personal communication). But, the apparent difference in the surface markers of the two types of T-lineage cells ($CD4^-8^-$ for $\gamma\delta$[20,21] and $CD4^+8^-$ or $CD4^-8^+$ for $\alpha\beta$) does not support this view. An alternative view of the $\gamma\delta$-bearing cells is that they define a T-cell subset other than CTL and T_H. A $\gamma\delta$-positive human cell line exhibiting a NK (natural killer) activity seems to exist[24].

As the putative $\gamma\delta$ heterodimer is expressed on the surface of the fetal thymocytes, one might also envision the possibility that such a structure could function as a receptor involved in the selection of a self MHC-restricted T-cell repertoire[25]. Before proposing a precise role of such a receptor in the expansion or deletion of specific thymocytes, however, several facts should first be established. These include the cell lineage relationship between the $\gamma\delta$- and $\alpha\beta$-bearing cells, the possible coexpression of the two receptors on the surface of a thymocyte subset and the functional ligands of the $\gamma\delta$ heterodimer.

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Note added in proof: Recently Lew *et al.* (*Science* **234**, 1401–1405; 1986) reported that a subpopulation of $CD4^-8^-$ adult thymocytes also express a γ-chain disulphide-bonded to a 45K component.

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A γ-chain complex forms a functional receptor on cloned human lymphocytes with natural killer-like activity

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We have recently derived from human fetal blood (25 wks) a series of cloned cell lines that were selected for their ability to kill the conventional natural killer (NK) target cell K562[1]. It was found that a fraction of these clones express CD3 proteins but not the monomorphic Ti $\alpha\beta$ determinant recognized by WT31 antibody[1]. One interleukin-2-dependent $CD3^+$ $WT31^-$ clone, termed F6C7, was used for immunization of mice to generate monoclonal antibodies directed at a potentially novel recognition receptor. It was shown that F6C7 cells, which transcribe Ti β but not Ti α genes, surface-express a clonotypic structure, termed NKFi[2]. Immunoprecipitations performed with anti-NKFi monoclonal antibody (mAb) indicated that the corresponding molecule is resolved in SDS-polyacrylamide gel electrophoresis (PAGE) as a single band of relative molecular mass ~85,000 (M_r ~85K). After reduction, a major band was detected at 44K and a faint band was present at 41K[2]. The present study was designed to characterize this structure. It was found that NKFi represents either two 44K disulphide-linked γ (TCR) chains, or possibly one γ chain associated to an additional undetected molecule, and that the 41K material corresponds to a partially glycosylated fraction of the γ protein. Anti-NKFi mAb both induces a specific autocrine proliferative response and blocks cytotoxic function, demonstrating that γ chains serve as functional receptor structures on subpopulations of normal human lymphocytes.

In a first series of experiments, we assessed whether NKFi is a glycosylated protein. After immunoprecipitation with anti-NKFi mAb, a fraction of the material was treated with endo-F, an enzyme which cleaves both high-mannose and complex glycans[3,4]. Figure 1*a* depicts a typical SDS-PAGE analysis of NKFi under non-reduced conditions (lane A), reduced conditions (lane B) and reduced conditions following endo-F digestion (lane C). As shown, we usually detected two major deglycosylation products at 41K and 34K respectively. These results indicated the presence of *N*-linked glycans on the native 44K molecule. To establish whether the minor 41K native structure (containing generally one tenth of the total number of specific counts) was also glycosylated, each band was individually extracted from preparative gels. Separate electrophoresis of heavy (Fig. 1*b*, lanes A and B) and light (Fig. 1*b*, lanes C and D) material was then performed either directly (lane A and C) or after treatment with endo-F (lane B and D). These experiments indicated that the final digestion product of each band resolved at the same M_r of ~34K suggesting that the

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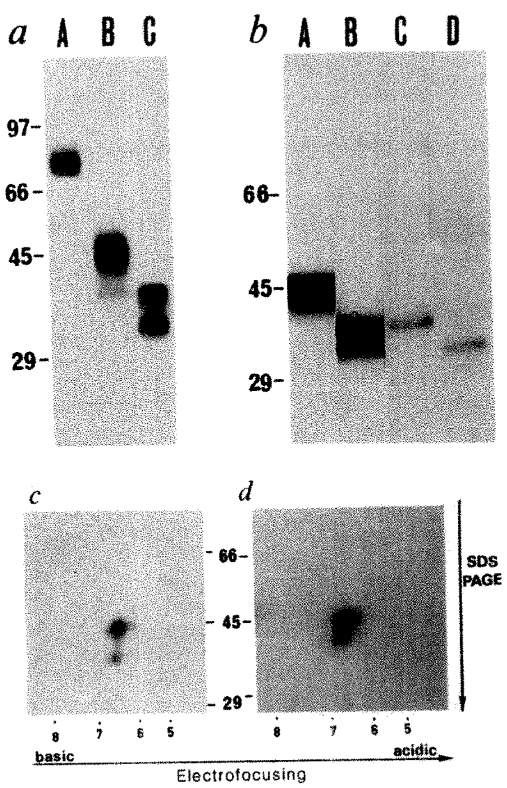

Fig. 1 Endo-F treatment of the NKFi molecule. *a*, lane A, NKFi, non-reduced conditions; lane B, NKFi reduced conditions (5% 2-mercaptoethanol (2ME); lane C, NKFi after endo-F treatment. *b*, lane A, extracted heavy chain; lane B, heavy chain treated with endo-f; lane C, extracted light chain; lane D, extracted light chain treated with endo-F. *c*, *d*, Two-dimensional gel electrophoresis of the NKFi molecule; two representative experiments.

Methods. *a*, Specific immunoprecipitation from ^{125}I-labelled F6C7 cells was followed by SDS-PAGE analysis as previously reported[2]. Endo-F treatment was performed as described[3]; samples were treated overnight at 37 °C with 0.2 units of endo-F (Boehringer). *b*, Heavy and light chains were cut and extracted from the gel in NH_4HCO_3 0.1 M buffer containing 0.05% SDS. After lyophilization, samples were dialysed to remove detergent and resuspended in 0.1 M Na_2HPO_4, 0.5% SDS, 1% 2-ME, 50 mM EDTA, 1% Triton X-100, buffer *p*H 6.1 containing protease inhibitors, before endo-F treatment. *c*, *d*, After immunoprecipitation from ^{125}I-labelled F6C7 cells, the NKFi molecule was reduced with 2-mercaptoethanol and applied at the basic end of the IEF gel. The *p*H gradient of the first dimension was obtained with a mixture of LKB ampholines (*p*H 3.5–10). The gel was equilibrated in second dimension buffer and laid on the top of an SDS 10% polyacrylamide gel for protein separation according to molecular mass. Markers shown are 29K, 45K and 66K M_r.

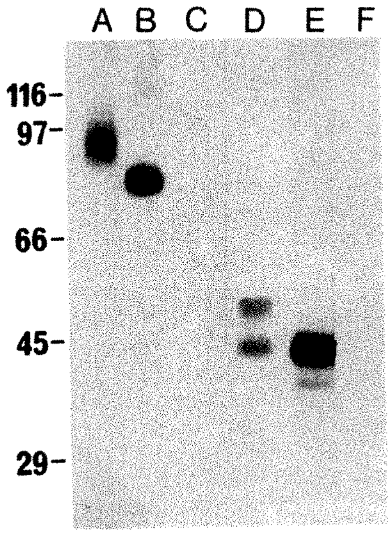

Fig. 2 Immunoprecipitations with βF1 antibody. Lanes A, D, βF1 antibody precipitations from a conventional Ti $\alpha\beta$ T-cell clone designated C15; lane B, E, anti-NKFi precipitations from F6C7 cells; lanes C, F, βF1 precipitations from F6C7 cells. SDS-PAGE was performed under either non-reduced (lanes A–C) or reduced conditions (lanes D–F).

fainter native 41K band may well correspond to a partially glycosylated form of the major 44K structure. To study the NKFi molecule further, the immunoprecipitated material was subjected to electrofocusing before SDS-PAGE analysis. As shown in Fig. 1*c*, *d* (two representative experiments) both the heavy and light structures had similar *p*I values (6.5–7). But the 44K band displays a greater charge heterogeneity with more acidic material compared to the 41K band. Together these experiments favoured the hypothesis that NKFi is a unique protein species surface-expressed very largely as a disulphide-linked dimer, with a small fraction of the polypeptide being not fully glycosylated. Note that it cannot be unequivocally excluded that NKFi contains one 44K structure plus an additional molecule that is not detected under these conventional experimental procedures.

Previous studies have demonstrated the existence of 3 distinct genes $\alpha^{5,6}$, β^{7-9} and γ^{10-14} encoding either established[15] or putative[16] T-cell receptor proteins. Because we could not detect Ti α transcripts in F6C7 cells, it was unlikely that the corresponding chain was part of the NKFi molecule. But a full size β message was found in the fetal lymphocytes; we therefore used anti-βF1 antibody (kindly provided by Dr M. Brenner), which is directed at a monomorphic determinant of β^{16}, to assess the potential surface expression of this protein. As shown in Fig. 2, βF1 mAb precipitated a 90K molecule from a control T-cell line under non-reduced conditions (lane A), resolving in 2 chains with respective relative molecular masses of 50K and 44K after reduction (lane D). This antibody failed to recognize any analogous structure in F6C7 cell lysates (Fig. 2, lanes C and F). To exclude the possibility that negative results simply reflect an alteration of βF1 epitope secondary to unusual association of β chains with a protein distinct from α, we performed Western blot experiments. F6C7 cell lysates were transferred to nitrocellulose membranes after electrophoresis under reduced conditions. Although specific bands were found on a variety of control T-cell lines, no signal at all was detected for F6C7 cells (not shown). We therefore conclude that β chains are not part of the NKFi molecule even though the fetal lymphocytes produce a full size β transcript which is probably not functional.

Further experiments were undertaken to investigate whether the clonotype expressed on F6C7 cells contains disulphide-linked γ chains. We firstly assessed whether γ genes were transcribed in the fetal lymphocytes. As shown in Fig. 3 (lane A), a full-size 1.5-kb γ message was detected. Thus, we synthesized a 20-amino-acid peptide corresponding to a sequence (residues 117–136) encoded by the first exon of $C_\gamma1$ constant region[16,17]; a series of rabbits were immunized with this peptide coupled to KLH, and specific antisera were used for immunoprecipitations. Results of these experiments were clear-cut: Fig. 4*a* shows that anti-C_γ sera precipitated from F6C7 lysates a structure resolving into two bands of 44K and 41K (Fig. 4*a*, lane B) which corresponds to the M_r of the protein detected with anti-NKFi under reducing conditions (Fig. 4*a*, lane A). Sequential immunoprecipitations were then conducted to confirm the identity between the molecule recognized by either anti-C_γ or anti-NKFi antibodies. It was found that preclearing of F6C7 lysates with anti-NKFi antibody resulted in the disappearance of the material recognized by the C_γ antisera

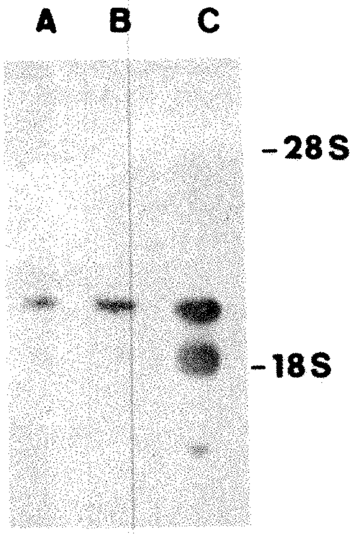

Fig. 3 Northern blot analysis of F6C7 cloned cell line with γ probe. Lane A, F6C7 cells: lane B, *p*H 28 cells, control T-cell clone known to transcribe γ chains (without apparent surface expression); lane C, JM cells, T cell leukaemia.

Methods. RNA was prepared from the cytoplasmic fraction of cells lysed in hypotonic buffer in the presence of Nonidet-P40. Total RNA was purified by two phenol-chloroform extractions, followed by two ethanol precipitations. The RNA preparations (5 μg per slot) were dissolved with water, heated at 50 °C for 1 h in the presence of 1 M glyoxal, 50% DMSO and run in a 1% agarose gel at 30 V for 5 h. The gel was soaked in 10 × SSC for 30 min and the RNA transferred on two Genescreen Plus nylon membranes placed on both sides of the gel. Hybridizations were carried out in 50% formamide, 5 × SSC, 1 × Denhardt's, 5% dextran sulphate wih 1 × 10⁷ counts per minute (c.p.m.) ml⁻¹ of ^{32}P-labelled RNA C γ probe at 65 °C for 16 h. This high specific activity probe was synthesized with T3 RNA polymerase and ^{32}P-UTP (800 Ci mmol⁻¹) using as template a *Bam*HI-*Acc*I C_γ fragment isolated from a PH28 cDNA library established in λgt10. The nylon membranes were washed twice in 2 × SSC, twice in 2 × SSC 1% SDS at 60 °C for 30 min and twice in 0.1 × SSC, 0.1% SDS at 65 °C for 30 min.

Fig. 4*a*, lane C). (It was not possible to perform the reverse xperiments because anti-C_γ sera did not precipitate the native olecule and specific binding of anti-NKFi antibody was abroated by the denaturation procedure.) To confirm that both the 4K and 41K bands contain the same protein species, they were ndividually extracted from gels and subsequently subjected to nti-C_γ immunoprecipitations. As shown in Fig. 4*b* (lane B and), both structures were individually reprecipitated by the pecific heteroantisera.

Together our results support the view that NKFi represents disulphide-linked γ-γ homodimer although, as already menioned, we cannot exclude the possibility that a γ-associated rotein has not been detected. In any case, it appears most likely

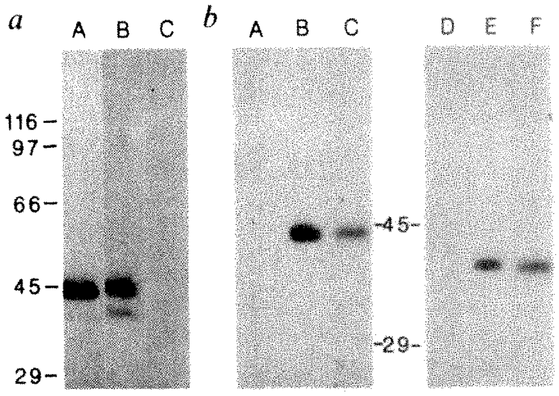

Fig. 4 *a*, Immunoprecipitating with C_γ antisera. Lane A, labelled F6C7 lysate immunoprecipitated with anti-NKFi antibody. SDS-PAGE was performed in the presence of 5% 2-mercaptoethanol. Lane B, immunoprecipitation with anti C_γ antisera following denaturation-renaturation procedures which include DTT treatment of the lysate[16]. Lane C, immunoprecipitation with anti-C_γ antisera under conditions described in lane B but after preclearing the lysates twice with anti-NKFi.*b*, Precipitation of both 44K and 41K NKFi chains. Lane A, heavy chain precipitated with rabbit preimmune sera; lane B, heavy chain precipitated with C_γ antisera; lane C, heavy chain; lane D, light chain precipitated with rabbit preimmune sera; lane E, light chain precipitated with C_γ antisera; lane F, light chain.

Methods. *a*, A 20-amino-acid peptide (residues 117-136) from the constant region of the γ chain[16,17] was synthesized using an Applied Biosystems apparatus according to the Merrifield method[18]. Following quality control by amino-acid analysis (LKB amino-acid analyser alpha) and fast atom bombardment mass spectrometry (VG400 mass spectrometer), the peptide was coupled to keyhole limpet haemocyanin (KLH). Various antisera were screened for specific reactivity with the immunizing peptide using appropriate ELISA assays; sera selected for further studies had specific reactivity in this experimental system at 1/10,000 dilution. Immunoprecipitations with γ-specific antisera were performed on F6C7 cell lysates using protein A-Sepharose beads after denaturation with SDS (final concentration 1%) reduction with DTT (2 mM) and subsequent heating (4 min at 100 °C) before addition of iodoacetamide (20 mM final concentration) as described by Brenner *et al.*[16]. *b*, Material precipitated from F6C7 cells with anti-NKFi antibody was subjected to SDS-PAGE and heavy and light chains were cut and extracted separately from the gel as described in Fig. 1. This material was prepared for immunoprecipitation as above and submitted to subsequent treatment with either anti-C_γ antisera (lanes B, E) or control preimmune sera (lanes A, D). Part of the extracted material was run in parallel as a control for separation of heavy (lane C) and light (lane F) chains. Note that the same amount of radioactivity was analysed in lanes B and C, or lanes E and F respectively.

that diversity exists in surface expression of γ chains. Indeed, the presence of this protein has been recently demonstrated on circulating lymphocytes from immunodeficient patients[16] as well as immature thymocytes[19]. But the CD3-associated material described by Brenner *et al.* was found to consist of two non-

Table 1 Proliferative response of F6C7 cells following stimulation by anti-T3 or anti-NKFi antibody

	Medium	rIL2	Anti-T3	Anti-T3 + rIL2	Anti-NKFi	Anti-NKFi + rIL2
F6C7	1,992	23,181	17,145	42,660	56,429	45,059
	±190	±3,282	±917	±1,749	±4,972	±2,636
PH.28	1,579	31,260	32,689	80,313	2,222	39,157
	±46	±2,639	±6,206	±4,751	±102	±1,015

Results represent the mean of ^{3}H-thymidine uptake in c.p.m. ± s.d. from triplicate cultures. 6 × 10⁴ cells per well were cultured in round-bottom plates for 3 d, including a 16-h pulse in the presence of either medium or antibodies coupled to BrCN Sepharose beads or rIL2 (final concentration 50 U ml⁻¹) or both. PH28 is a CD3⁺ CD4⁺ inducer control clone specific for diphtheria toxoid. Note that anti-NKFi induces a strong proliferation without the addition of exogenous interleukin-2. This proliferation was not enhanced if interleukin-2 was added in the experimental system demonstrating that the specific response developed through an autocrine pathway.

disulphide-linked molecules including γ and a putative additional structure designated δ[16]. Thus, future experiments will have to dissect heterogeneity in membrane organization of these novel receptors and elucidate its biological relevance in T-lymphocyte differentiation.

As described in this study, NKFi structure is defined by both constant region antisera and a monoclonal antibody specific for a clonotypic determinant. This latter reagent recognizes the native molecule on viable cells and has been shown[2] to block cytotoxicity of the F6C7 clone. Furthermore, experiments performed here (Table 1) show that anti-NKFi coupled to Sepharose beads induces a specific signal for autocrine proliferation, which is probably transduced through CD3 proteins given the similar effect of anti-CD3 antibody (Table 1). It can therefore be concluded that γ chains actually serve as functional receptor structures on subpopulations of human lymphocytes.

The unique CD3$^+$ WT31$^-$ phenotype which appears to be predictive of γ chain surface expression was originally described[1] in studying human fetal lymphocytes selected for their ability to display 'natural killer-like' function. We have now generated a number of CD3$^+$ cloned cell lines derived from either thymus or adult normal peripheral blood which were characterized because they do not react with WT31 monoclonal antibody. Each of these later IL-2-dependent CD3$^+$ WT31$^-$ clones kill genetically unrelated tumour cell lines. Thus, further experiments are necessary to confirm whether surface expression of γ chains consistently correlates with such a non-MHC restricted cytotoxic function and, perhaps more importantly, to establish the potential role of this structure in target cell recognition.

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Independent phosphatidylinositol synthesis in pituitary plasma membrane and endoplasmic reticulum

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Phosphatidylinositol (PtdIns), the most abundant phosphoinositide, is the precursor of phosphatidylinositol 4-monophosphate which is converted to phosphatidylinositol 4,5-bisphosphate, the lipid hydrolysed as an early step in signal transduction by many stimuli[1-3]. It is generally thought that a single enzyme in the endoplasmic reticulum, PtdIns synthase (CDP-diglyceride:*myo*-inositol 3-phosphatidyltransferase, EC 2.7.8.11), is responsible for PtdIns synthesis and that newly synthesized PtdIns is transported to the plasma membrane by exchange proteins[4]. Several investigators have proposed that there are two functionally distinct pools of PtdIns, one responsive to stimulation and the other not[5-8], and that the stimulus-responsive pool may be synthesized at a different site within the cell, perhaps within the plasma membrane. Indeed, it was suggested that there is PtdIns synthase activity in plasma membrane isolated from rat liver[9]. GH$_3$ rat pituitary tumour cells are an excellent model system to study stimulation of phosphoinositide metabolism by thyrotropin-releasing hormone (TRH)[10]. Conversion of PtdIns to polyphosphoinositides[11] and TRH (and GTP)-activated phosphoinositide hydrolysis[12,13] are known to occur in plasma membrane isolated from GH$_3$ cells. Here we report that PtdIns synthase activity in the plasma membrane of GH$_3$ cells is distinct from that present in the endoplasmic reticulum. The plasma membrane PtdIns synthase may be responsible for a portion of PtdIns re-synthesis that occurs during cell stimulation.

Figure 1 illustrates the profile of activity of *myo*-[^{3}H]inositol incorporation into PtdIns in GH$_3$ cell membranes fractionated on a continuous sucrose density gradient. There are two peaks of activity, corresponding by marker enzyme analysis to the plasma membrane and endoplasmic reticulum fractions. More than 98% of the ^{3}H-radioactivity incorporated into lipid was in PtdIns, which was membrane-bound as it was recovered in the pellet after centrifugation at 100,000g for 60 min. Using the standard assay buffer, the specific activity in the plasma membrane was approximately twice that in the endoplasmic reticulum. In contrast, as reported in other cell types[9,14], activity in the endoplasmic reticulum causing incorporation of *myo*-inositol into PtdIns could be increased several-fold above that in plasma membrane by using different assay conditions, for example by increasing the concentration of Mn^{2+} or substrates (Fig. 2*c*). Nevertheless, because the total protein recovered in the two fractions was the same (29 ± 5.4 versus 30 ± 2.6 μg per 10^7 cells), the total activity that causes *myo*-inositol incorporation into PtdIns, when assayed in a buffer without Mn^{2+} or in a buffer that more closely simulates cytosolic conditions (Fig. 2 legend), was greater in plasma membrane than in endoplasmic reticulum.

In addition to PtdIns synthase, enzyme-mediated base exchange, which does not lead to net synthesis of PtdIns and can occur in the absence of CDP-diglyceride, can be responsible for incorporation of *myo*-[^{3}H]inositol into PtdIns[15]. The contribution of base exchange, incorporation in the absence of added CDP-diglyceride, to the activity measured in plasma membrane and endoplasmic reticulum was $21 \pm 9\%$ and $1.6 \pm 0.6\%$, respectively (Fig. 2*a*); these may be overestimations because endogenous CDP-diglyceride may have been present in the membrane fractions. Evidence that supports the notion that *myo*-[^{3}H]inositol incorporation in the absence of CDP-diglyceride was by base exchange and in the presence of CDP-diglyceride was mainly by *de novo* synthesis catalysed by PtdIns synthase, was obtained in experiments in which GH$_3$ cell membranes were incubated with *myo*-[^{3}H]inositol without or with CDP-diglyceride and then a large excess of unlabelled *myo*-inositol was added. The *myo*-[^{3}H]inositol incorporated into PtdIns in the absence of CDP-diglyceride was readily chased by unlabelled *myo*-inositol, whereas that incorporated in the presence of CDP-diglyceride was not affected during subsequent incubation with unlabelled *myo*-inositol (data not shown).

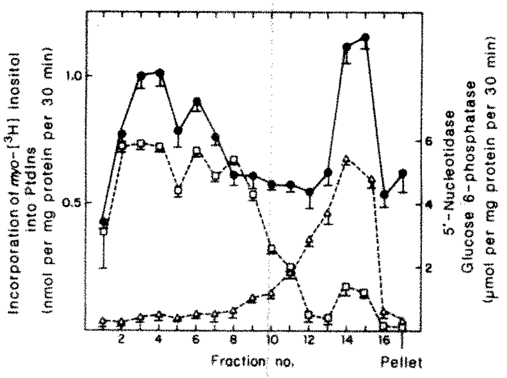

Fig. 1 Distribution of PtdIns synthase (●), 5′-nucleotidase (□) and glucose 6-phosphatase (△) specific activities in membranes isolated from GH_3 cells after centrifugation on a continuous sucrose density gradient.

Methods. GH_3 cells were grown as monolayer cultures in Ham's F-10 medium supplemented with 15% horse serum and 2.5% fetal calf serum at 37 °C and collected with 0.02% EDTA[24]. Lysis buffer (0.5 mM dithiothreitol, 1 mM EGTA, 0.5 mM sodium bicarbonate and 10 mM HEPES, *p*H 7.9) was added (~4 to 5 times the cell pellet volume) and the cells were suspended and simultaneously lysed by incubating at 37 °C for 4–5 min, followed by addition of 10 volumes of ice-cold lysis buffer and rapid mixing on a vortex mixer for 0.5 min at 0–4 °C. The homogenate was centrifuged at 800*g* for 10 min to eliminate nuclei and cell debris. The supernatant was centrifuged again at 100,000*g* for 1 h and the pellet was layered on top of a continuous sucrose density gradient, 25–50% (w/v), and then centrifuged at 100,000*g* in a swinging-bucket rotor for 1 h. The fractions were washed with lysis buffer and assayed immediately. PtdIns synthase activity was measured as the incorporation of *myo*-[^{3}H]inositol into PtdIns in the presence of CDP-diglyceride. The assay buffer contained 100 mM Tris-HCl, *p*H 7.5, 1 mM EGTA, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 0.1 mM CDP-dipalmitoyl glycerol (Sigma), 0.1 mM *myo*-[^{3}H]inositol ($\sim 1 \times 10^5$ d.p.m. $nmol^{-1}$, NEN) and membrane protein (20–50 μg) in a total volume of 0.05 ml. CDP-diglyceride was added after dispersal by brief sonication. Similar results were obtained when the activities were measured in buffer containing 100 mM KCl and 10 or 20 mM NaCl in the presence or absence of Mn^{2+}. The reaction was started by the addition of membrane suspensions, and was incubated at 37 °C for 30 min. Under these conditions, the rate of formation of PtdIns was constant for at least 60 min. The reactions were terminated by adding 1 ml of chloroform/methanol/concentrated HCl (100:100:1, v/v/v) and the phases separated with 0.25 ml of 10 mM EDTA. The upper phase was re-extracted with pre-equilibrated lower phase, and combined with the lower phases. The extracted lipid was washed with 1 ml pre-equilibrated upper phase. PtdIns was isolated by thin-layer chromatography on silica gel 60 using a solvent system of chloroform/methanol/acetic acid/water (50:30:8:4, v/v/v/v). Most (>98%) of the radioactivity in the lipid fraction co-migrated with authentic PtdIns. Marker enzyme analyses were performed using 5′-nucleotidase[25] and (Na^+-K^+)ATPase[27] as markers for plasma membrane, succinate dehydrogenase[28] for mitochondria, and glucose 6-phosphatase[29] and NADH dehydrogenase[30] for endoplasmic reticulum; only 5′-nucleotidase and glucose 6-phosphatase are shown. Although not specifically assayed, it is probable that the plasma membrane fractions were contaminated with membranes from Golgi apparatus. Protein was determined according to the method of Lowry *et al.*[31] using bovine serum albumin (BSA) as standard.

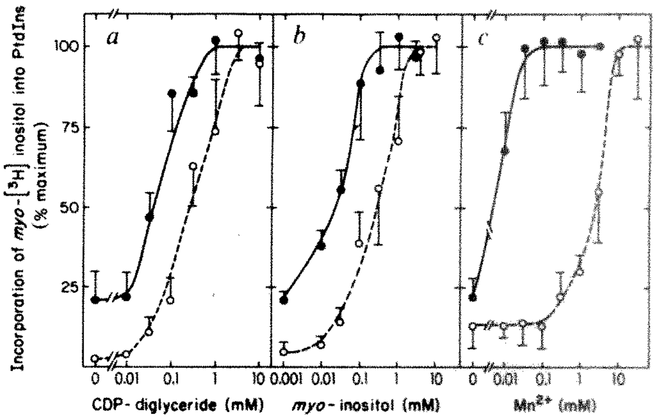

Fig. 2 Effects of *a*, *myo*-inositol; *b*, CDP-diglyceride; and *c*, Mn^{2+} on the incorporation of *myo*-[^{3}H]inositol into PtdIns in plasma membrane (●) and endoplasmic reticulum (○) isolated from GH_3 cells.

Methods. GH_3 cells were grown, collected and lysed, and a post-nuclear fraction obtained, as in Fig. 1. The resulting pellet was resuspended in lysis buffer that was layered on a discontinuous sucrose gradient consisting of 30, 36, 41, 45 and 50% (w/v) sucrose in lysis buffer. The gradient was centrifuged at 100,000*g* for 1 h yielding five narrow bands at the interfaces that were collected separately. The upper and lower two bands were collected as the plasma membrane and endoplasmic reticulum fractions, respectively. Incorporation of *myo*-inositol into PtdIns in the plasma membrane or endoplasmic reticulum fractions was measured as in Fig. 1, except that the concentration of CDP-diglyceride, *myo*-[^{3}H]inositol, or $MnCl_2$ was varied.

Hence, most of the activity that caused incorporation of *myo*-[^{3}H]inositol into PtdIns measured in plasma membranes and endoplasmic reticulum was caused by PtdIns synthase.

The PtdIns synthase activity in plasma membrane is different from the activity in endoplasmic reticulum (Fig. 2). The apparent K_m values for CDP-diglyceride and *myo*-inositol, calculated from double reciprocal plots, were different. For *myo*-inositol, K_m was 0.067 ± 0.009 and 0.26 ± 0.045 mM ($P < 0.001$) for plasma membrane and endoplasmic reticulum activities, respectively, and for CDP-diglyceride, K_m was 0.040 ± 0.016 and 0.33 ± 0.12 mM ($P < 0.01$) for plasma membrane and endoplasmic reticulum activities, respectively. A more marked difference was found in the effect of Mn^{2+} in enhancing PtdIns synthase activities in plasma membrane and endoplasmic reticulum. For Mn^{2+}, K_m was 0.015 ± 0.005 and 3.3 ± 1.3 mM ($P < 0.005$) for plasma membrane and endoplasmic reticulum, respectively. In contrast, the effect of Mg^{2+} in stimulating PtdIns synthase activity (half-maximal stimulation at 0.03–0.1 mM Mg^{2+}), of *p*H (optim ~7.5), and of Ca^{2+} in inhibiting activity (half-maximal nhibition at 0.1 mM free Ca^{2+} and maximal inhibition of >80% at 1 mM free Ca^{2+}) were similar. These data demonstrate that PtdIns synthase activities in plasma membrane and endoplasmic reticulum are distinct; they do not show that there are two PtdIns synthases because the same enzyme acting in different environments could display different properties.

Phosphoinositide kinases are present within plasma membranes[11,16] and recent experiments using membranes isolated from several cell types[17,18], including GH_3 cells[12,13], have demonstrated stimulus-induced, phospholipase-C-mediated phosphoinositide hydrolysis, which is dependent on guanine nucleotides[19]. These findings confirm the suggestion that at least part of phosphoinositide degradation in intact cells occurs in the plasma membrane. However, experiments performed to show phosphoinositide degradation[20] or re-synthesis[21] in plasma membrane after stimulation of intact cells have been inconclusive. We attempted to discover the location of enhanced re-synthesis of PtdIns in intact GH_3 cells during TRH stimulation by monitoring incorporation of *myo*-[^{3}H]inositol. When intact cells were incubated in buffer containing micromolar to millimolar concentrations of *myo*-[^{3}H]inositol for 10–45 min in the presence or absence of TRH, we were unable to show any increase in incorporation into plasma membrane (data not shown). Because the loss of unesterified *myo*-inositol from cells was slow (unpublished data), we considered the possibility that the inability to measure stimulated incorporation of *myo*-[^{3}H]inositol was caused by insufficient depletion of endogenous *myo*-inositol. We therefore attempted to deplete cellular *myo*-inositol by transiently permeabilizing the cells in *myo*-inositol-free buffer and then resealing them in the presence of *myo*-[^{3}H]inositol; cells made permeable and then resealed are responsive to TRH[22]. In resealed cells incubated with *myo*-[^{3}H]inositol, specific ^{3}H-radioactivity in PtdIns was similar in plasma membrane and in endoplasmic reticulum (Table 1). TRH caused 2.4- and 2.2-fold increases in specific ^{3}H-radioactivity in plasma membrane PtdIns and endoplasmic reticulum PtdIns, respectively. Because the plasma membrane content of PtdIns was similar to that of the endoplasmic reticulum, the rapid and similar increases in specific ^{3}H-radioactivity in PtdIns stimulated by TRH in both membrane fractions make it unlikely that most

Table 1 Effect of TRH on the specific ^{3}H-radioactivity in PtdIns in plasma membrane and endoplasmic reticulum isolated from permeabilized and resealed GH$_3$ cells

	Specific ^{3}H-radioactivity in PtdIns (d.p.m. nmol^{-1})	
	Control	TRH
Plasma membrane	887 ± 13	$2{,}110 \pm 156$
Endoplasmic reticulum	883 ± 103	$1{,}920 \pm 241$

Cells were permeabilized and resealed by the method of Snowdowne and Borle[22]. In brief, cells were collected and washed as described in Fig. 1 legend and resuspended in 2 ml hypotonic buffer containing 3 mM HEPES, *p*H 7.4, and 3 mM ATP at 4 °C. After 3 min, 1 μM *myo*-[^{3}H]inositol was added followed by 0.14 ml of a solution containing 2 M KCl and 3 mM HEPES. After 15 min, the cells were washed and resuspended in a buffer consisting of 135 mM NaCl, 4.5 mM KCl, 0.5 mM MgCl$_2$, 1.5 mM CaCl$_2$, 5.6 mM glucose and 10 mM HEPES, *p*H 7.4 at 37 °C. After an additional 15 min, cells were incubated without (control) or with 1 μM TRH for 5 min. The membrane fractions were isolated as described in Fig. 1 legend and the incorporation of ^{3}H-radioactivity and the phosphorus content of PtdIns were determined[24]. PtdIns was isolated by two-dimensional thin-layer chromatography[26]. The subcellular distribution of PtdIns, measured as stable phosphorus[24], was 39±6.6% in plasma membranes, 25±6.1% in the endoplasmic reticulum and 36±9.0% in mitochondria.

plasma membrane PtdIns is synthesized in the endoplamic reticulum and then transported to the plasma membrane.

These data demonstrate that PtdIns is synthesized within plasma membrane as well as endoplasmic reticulum of GH$_3$ cells and provide initial evidence that during stimulation of intact cells by TRH both sites are active. Although our data were derived from experiments that used a rat pituitary tumour cell line, our findings may be applicable to all cells that respond to stimuli that use phosphoinositide hydrolysis as an early step in signal transduction. The findings reported here are important in understanding phosphoinositide metabolism during cell stimulation by showing that rapid, vectorial flow of newly synthesized PtdIns from the endoplasmic reticulum to a stimulus-responsive phosphoinositide pool[5-8] is not needed. Because recent findings that stimulation of PtdIns hydrolysis is persistent whereas that of phosphatidylinositol 4,5-bisphosphate is transient[23-25] are compatible with a direct role for PtdIns hydrolysis in the prolonged actions of stimuli, it is possible that hydrolysis of PtdIns within the plasma membrane is the mechanism of prolonged generation of diacylglycerol for activation of protein kinase C. Indeed, our observations suggest that a stimulus-responsive pool of phosphoinositides is present within the plasma membrane and that both hydrolysis and re-synthesis of this pool of phosphoinositides occurs in it.

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Coevolution of codon usage and transfer RNA abundance

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The use of synonymous codons is strongly biased in the bacterium *Escherichia coli* and yeast, comprising both bias between codons recognized by the same transfer RNA and bias between groups of codons recognized by different synonymous tRNAs (reviewed in ref. 1). A major determinant of the second sort of bias is tRNA content, codons recognized by abundant tRNAs being used more often than those recognised by rare tRNAs, particularly in highly expressed genes, probably owing to selection at the level of translation against codons recognized by rare tRNAs[1-3]. Conversely, codon usage is likely to exert selection pressure on tRNA abundance[4]. Here I develop a model for the coevolution of codon usage and tRNA abundance which explains why there are unequal abundances of synonymous tRNAs leading to biased usage between groups of codons recognized by them in unicellular organisms.

Consider first the evolution of codon usage with tRNA abundance held constant. At a particular coding position suppose that there are two groups of synonymous codons, A$_1$ and A$_2$, recognized by two isoaccepting tRNAs. Suppose that the second tRNA is more rare than the first so that A$_2$ has relative fitness $1-s$ compared with A$_1$($s>0$), and let u be the mutation rate between A$_1$ and A$_2$, assumed the same in each direction. Because s is likely to be very small, the relative frequencies of A$_1$ and A$_2$, denoted by p and $q=1-p$, will be determined by mutation-selection balance. Ignoring non-synonymous mutations and drift, the equilibrium frequency of A$_2$ is

$$q=\frac{1}{2}+\frac{u}{s}-\frac{1}{2}\left[4\left(\frac{u}{s}\right)^2+1\right]^{1/2} \tag{1}$$

(If A$_2$ is at a selective advantage so that $s<0$, the sign of the last term becomes positive.) This model, assuming independent selection at each coding position, seems more plausible[5] than Kimura's model[4] which supposes that there is an optimum frequency of A$_1$ codons in the genome.

Availability of tRNA affects translation time[6-9] and it has been suggested[6] that the time, t, taken to translate a codon can be expressed as

$$t=\theta/C+\tau \tag{2}$$

where θ is the mean waiting time before the arrival of a tRNA plus the time taken to test it, C is the relative abundance of the matching tRNA (scaled to sum to unity, so that $1/C$ is the average number of tRNAs tested before a match is found), and τ is the time taken for transpeptidation and translocation (likely to be small compared with the first term[6-10]). Other factors affecting codon usage, such as contextual constraints[11] and mRNA secondary structure are ignored in equation (2), on the grounds that they are of minor importance compared with tRNA

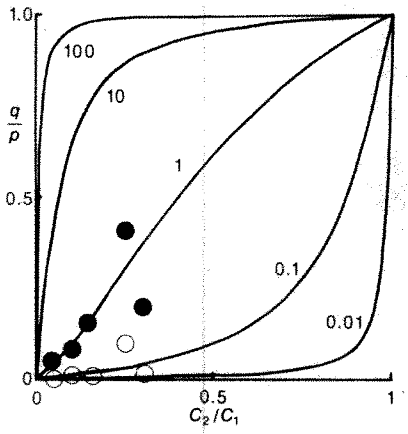

Fig. 1 Predicted codon usage (q/p) plotted against amount of tRNA (C_2/C_1) at different levels of gene expression (inversely proportional to u/k, shown on each curve) when codon usage evolves at fixed tRNA abundance to an equilibrium mutation-selection balance. Points are observed data[1] in *E. coli* for the strongly expressed gene *ompA* (○) and the weakly expressed *lac* repressor gene (●).

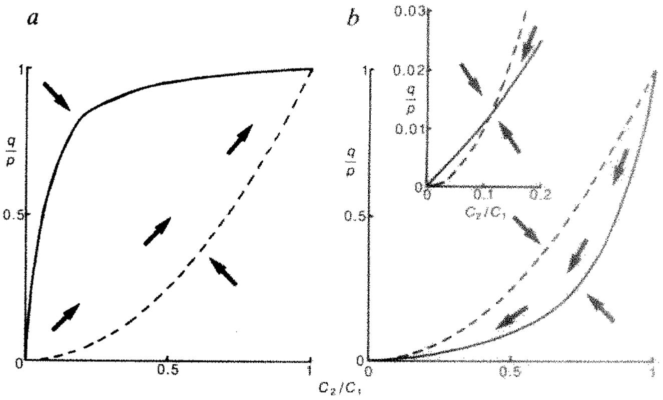

Fig. 2 Predicted codon usage (q/p) plotted against amount of tRNA (C_2/C_1) for $u/k=(a)$ 10, (b) 0.1. Solid curve shows evolution of codon usage with fixed tRNA abundance (see Fig. 1). Dashed curve shows evolution of tRNA abundance with codon usage fixed. Inset in (b) is an enlargement near the origin. Arrows show the direction of movement, which has an upward or downward component according to whether the point is below or above the solid curve, and a left or right component according to as it is to the right or left of the dashed curve.

abundance[6] and will average out when comparison is made between groups of codons recognized by different tRNAs.

An increase in translation time may reduce fitness either directly, because rapidity of clonal division is a component of fitness, or indirectly through the extra energy needed to test and reject non-cognate tRNAs. In either case, if t_1 and t_2 are the average times taken to translate codons in groups A_1 and A_2, we may suppose that

$$s=\alpha(t_2-t_1)=k\left(\frac{C_1}{C_2}-1\right) \tag{3a}$$

$$k=\alpha\theta/C_1 \tag{3b}$$

where α depends on the degree of expression of the gene. Because C_1, the relative abundance of the major tRNA, is about the same for all amino acids[1], k can be regarded as a constant within a gene and equations (1) and (3) can be used to calculate q/p as a function of C_2/C_1 given the single parameter u/k. Theoretical curves for different values of u/k are shown in Fig. 1. If the forward and backward mutation rates are unequal, say u_1 from A_1 to A_2 and u_2 the other way, similar curves would be obtained starting at the origin but meeting at $q/p=u_1/u_2$ when $C_2/C_1=1$.

Ikemura[1] has plotted tRNA usage against amount of tRNA for different genes of *E. coli* and yeast; for each amino acid both variables were normalized to 1 for the most abundant tRNA. These variables are directly comparable with the theoretical variables plotted in Fig. 1, and the observed data are shown for comparison for a weakly expressed gene (the *lac* repressor, ***lacI***) and a highly expressed gene (the outer membrane protein gene, ***ompA***) in *E. coli*. The results for ***lacI*** lie approximately on the curve predicted for $u/k=1$, whereas those for *ompA* lie on one of the markedly convex curves (indicating a much stronger bias) predicted when $u/k \ll 1$, consistent with the expectation that k increases with the degree of gene expression.

It is of interest to see whether a very simple model relating fitness to translation time can generate a selection pressure of the right order of magnitude. Suppose that the ith codon in the genome takes t_i seconds to translate and that this codon is used r_i times during one mitotic division, so that r_i molecules of its gene product are made. Under optimal growth conditions, the time taken for a division is $T=\sum r_i t_i/R$, where R is the number of ribosomes. Under these conditions, the fitness of an organism (W) can be taken as the reciprocal of the division time, $W=1/T$. Hence for a codon used r times, $\alpha=r/RT$ (see equation (3)) and $k=r\theta/C_1RT$. For *E. coli*, doubling twice an hour[10], $\theta/C_1 \approx$ translation time per codon $=0.061$ s, $R=9{,}400$, so that $k=3.6r\times10^{-9}$. Estimates of r are 40 and 3×10^4 for *lacI*[3] and *ompA*[1], giving $k\approx10^{-7}$ and 10^{-4} respectively. For *lacI*, Fig. 1 suggests that $k\approx u$, the mutation rate per site, which is probably less than 10^{-7}. Thus the model has overestimated the selection pressure, which is perhaps not surprising in view of its crudity, but the calculation shows that the direct effect of translation time on fitness in a clonal organism can generate enough selection to account for the bias in codon usage observed in Fig. 1.

Now consider the evolution of tRNA abundance with codon usage held fixed. Thus, suppose that q is fixed but that C_2 can evolve, with C_1+C_2 held constant; this constraint is appropriate as we want to determine the optimal allocation of resources between the two tRNAs. Consider a mutant which increases C_2 to C_2+d and decreases C_1 to C_1-d, where d is small. From equation (2) the change in the translation time is $\Delta t_1=\theta d/C_1^2$ for an A_1 codon and $\Delta t_2=-\theta d/C_2^2$ for an A_2 codon. From equation (3) the selection coefficient against the mutant is

$$\Delta t_1 \sum_1 \alpha+\Delta t_2 \sum_2 \alpha=dC_1\left(C_1^{-2}\sum_1 k-C_2^{-2}\sum_2 k\right) \tag{4}$$

where $\sum_1$ and $\sum_2$ denote summation over A_1 and A_2 codons respectively. Setting (4) equal to zero at equilibrium, $(C_2/C_1)^2=\sum_2 k/\sum_1 k$.

To take into account differences in codon usage and in the selection intensity k between poorly and highly expressed genes, divide genes into groups with different levels of expression and suppose that a fraction π_j of the codons belong to the jth group and that in this group $k=k_j$ and the relative frequencies of A_1 and A_2 are p_j and q_j respectively. Then $\sum_2 k/\sum_1 k=\bar{q}/\bar{p}$ where $\bar{p}$ and $\bar{q}$ are the weighted averages $\bar{p}=\sum_j w_j p_j$, $\bar{q}=\sum_j w_j q_j$ with $w_j=\pi_j k_j/\sum_j \pi_j k_j$. Hence

$$(C_2/C_1)^2=\bar{q}/\bar{p} \tag{5}$$

This result depends on the assumption that selection pressure is inversely proportional to tRNA abundance.

We can now investigate the coevolution of codon usage and tRNA abundance by combining the separate analyses. Suppose first that all genes have the same degree of expression. Figure 2 shows the superposition of the curve for the evolution of codon usage (solid, reproduced from Fig. 1) and the curve for the evolution of tRNA abundance (dashed, from equation (5)). Two values of u/k, representing different levels of gene expression, are illustrated. In each case the equilibria under coevolution are the points of intersection of these two curves; their stability can be inferred from the arrows representing the dynamics of the system. When $u/k=10$ (weak gene expression, Fig. 2*a*) there are two equilibria, at (0, 0) and (1, 1), of which the first is unstable and the second stable; we predict equal tRNA abundances and little bias in codon usage. When $u/k = 0.1$ (strong gene expression, Fig. 2*b*) there are three equilibria, at (0, 0), (0.113, 0.013) and (1, 1), of which the first and third are unstable and the second stable; we expect to see unequal tRNA abundances and strong bias in codon usage.

The solid curve lies above the dashed curve near the origin for all values of u/k because its slope at the origin is u/k whereas the dashed curve is horizontal. Whether the system behaves as shown in Fig. 2*a* or *b* depends on whether the solid curve approaches (1, 1) from above or below the dashed curve. Evaluating the slopes of the curves at this point shows that this depends on whether u/k is greater or less than 0.25. When u/k is small the stable equilibrium is approximately $(u/k, (u/k)^2)$.

With variable gene expression, we must plot $\bar{q}/\bar{p}$ against C_2/C_1. Equations (1) and (3) allow q_j to be calculated in each category of gene expression determined by k_j, whence $\bar{q}/\bar{p}$ can be calculated to give the solid curve; the dashed curve is unchanged. The argument used in the previous paragraph shows that the system behaves as in Fig. 2*a* or *b* according as $u/\bar{k}$ is greater or less than 0.25. Note that $\bar{k}$ is a weighted average, $\bar{k} = \sum_j w_j k_j$, so that strongly expressed genes are highly influential in determining the behaviour of the system.

This result provides a possible explanation for the existence of highly biased codon usage associated with unequal tRNA abundances in unicellular organisms in which rapid clonal division is an important component of fitness so that there is strong selection pressure to minimize translation time. To obtain biased codon usage under this model the weighted mean selection pressure per nucleotide, $\bar{k}$, in response to tRNA abundance must exceed both the mutation rate per nucleotide, u, and the reciprocal of the population size[12], otherwise codon usage is largely determined by mutation pressure. Data on the relationship between codon usage and tRNA abundance[1] (Fig. 1) show that $u/\bar{k}$ is small in both *E. coli* and yeast, so that coupled with their large population size they meet the requirements for biased codon usage. Multicellular organisms are likely to have both lower selection pressure at the level of translation and smaller population size, consistent with the conclusion that their codon usage is heavily influenced by mutation pressure[1].

Several simplifying assumptions have been made in developing the model, but it seems unlikely that the conclusions would be qualitatively changed by relaxing them, except for the assumption in obtaining equation (5) that selection pressure is inversely proportional to tRNA abundance.

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Structure of D-ribulose-1,5-bisphosphate carboxylase/oxygenase from *Alcaligenes eutrophyus* H16

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RuBPCase, D-ribulose-1,5-bisphosphate carboxylase/oxygenase (EC4.1.1.39) is the key enzyme of the reductive pentose phosphate cycle. Because of its biological significance, many structural studies on a number of plant and bacterial RuBPCases[1] have been undertaken, including the enzyme isolated from the autotrophic hydrogen-oxidizing bacterium *Alcaligenes eutrophus* H16 (refs 2–6). Although both the higher plant enzyme and the *A. eutrophus* enzyme consist of eight large and eight small subunits (L_8S_8), no model describing the quaternary structure is generally accepted. Here we present a model for the *A. eutrophus* RuBPCase derived from X-ray crystallography of three-dimensional (3D) crystals, and electron microscopy and image analysis of two-dimensional (2D) crystals of the enzyme. The X-ray electron density of RuBPCase in the presence of HCO_3^-, Mg^{2+}, and the transition state analogue 2-carboxyarabinitol-1,5-bisphosphate (CABP) shows an L_8S_8 molecule in which the L_4S_4 half molecules have local 4-fold symmetry (C4). The local 4-fold axes of the two L_4S_4 halves do not coincide but are shifted by 36 Å and are related by a crystallographic 2-fold axis perpendicular to and between the local 4-fold axes. Electron microscope data of the enzyme without CABP, which can be perfectly modelled using the X-ray densities, do not show this shift and the low-resolution point group of the molecules in the 2D crystals is D4. Both structures are presented.

A variety of approaches to analysing the major structural aspects of the tobacco and the *A. eutrophus* RuBPCase have led to the consensus that the molecule has an L_8S_8 stoichiometry, a diameter of around 125 Å and a height of around 100 Å. These studies include electron microscopy of negatively stained[2,7] and antibody-labelled[4] single molecules, and thin[8] and sectioned[5,7] 3D crystals, as well as preliminary X-ray crystallographic work[5,7–9]. Some crystal forms have 1/4 molecule per asymmetric unit[7] indicating a minimal point group symmetry D2; others have point group symmetry C4[8] or even D4[9]. Problems associated with X-ray studies on *A. eutrophus* RuBPCase include, for example, twinned crystals of apparent space group $P4_22_12$[5,6]. The current models for L_8S_8 RuBPCase from tobacco and *A. eutrophus* show 4-fold rotational symmetry[2,8,9], but differ in spatial arrangement of the subunits.

The enzyme exists in two different activation states: high activity is associated with the presence, and inactivity with the absence, of HCO_3^- and Mg^{2+} ions[10]. The change in activation state is paralleled by a change in enzyme configuration[11] and in sedimentation behaviour of the enzyme (active: 14.3S; inactive: 17.5S)[10].

For X-ray diffraction studies, large single crystals of *A. eutrophus* RuBPCase were grown in the presence of HCO_3^-, Mg^{2+} and CABP[6]. The space group $C222_1$ and unit cell constants $a = 158.8$ Å, $b = 159.6$ Å, $c = 201.2$ Å imply that the asymmetric unit contains half an L_8S_8 molecule; the other half is related by a crystallographic 2-fold rotation axis. X-ray diffraction data of

Table 1 Quality of SIR phases

Resolution (Å)	16.67	12.50	10.00	8.33	7.14	6.25	5.65	5.00	Total
Figure of merit	0.67	0.58	0.70	0.63	0.59	0.55	0.47	0.44	0.53
Phasing power*	1.71	1.73	1.82	1.72	2.03	2.16	1.95	1.73	1.88
Anomalous dispersion†	2.13	1.83	2.01	1.78	1.46	1.33	1.29	1.23	1.32

* Phasing power = r.m.s. (f_H/E), where f_H is the calculated heavy atom structure factor amplitude and E is the closure error.

† Anomalous dispersion is the anomalous dispersion difference divided by the estimate of the error.

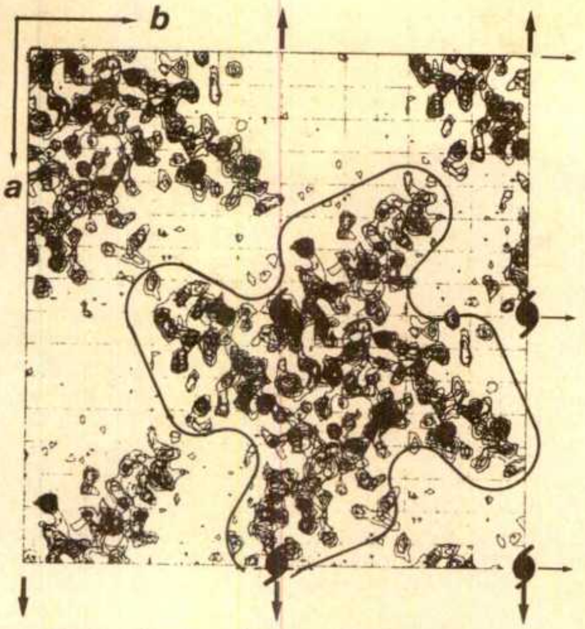

Fig. 1 Electron density sections parallel to the *a*, *b* plane. Crystallographic symmetry axes are indicated. Only one half of the unit cell, in the *c* direction, is shown. Data were collected by the rotation method. The native and $HgCl_2$-derivatized 3.2-Å data sets gave $^*R_{\text{merge}}$ values of 0.129 for 36,933 reflections (native) and 0.158 for 36,129 reflections (derivative), where $^*R_{\text{merge}} = \sum(I(i) - <I>)/I(i)$, where $I(i)$ is the intensity of an individual measurement and $<I>$ is the average value. The heavy atom positions were determined by difference Patterson methods. Once the heavy atom coordinates were obtained and refined (least squares and phase refinement), SIR phases were calculated (Table 1) and used to compute a 5-Å-resolution electron density map. Then, solvent flattening, map averaging, and phase combination procedures were used which converged at an *R* factor of 16% for agreement between structure amplitudes calculated from the modified electron density map and observed data, an average figure of merit of 0.82, and an accumulated phase shift of 58.1°.

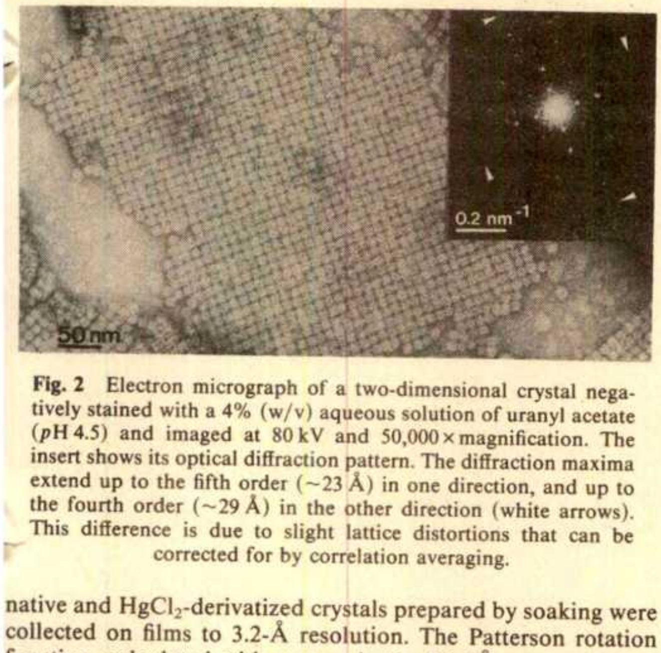

Fig. 2 Electron micrograph of a two-dimensional crystal negatively stained with a 4% (w/v) aqueous solution of uranyl acetate (*p*H 4.5) and imaged at 80 kV and 50,000× magnification. The insert shows its optical diffraction pattern. The diffraction maxima extend up to the fifth order (~23 Å) in one direction, and up to the fourth order (~29 Å) in the other direction (white arrows). This difference is due to slight lattice distortions that can be corrected for by correlation averaging.

native and $HgCl_2$-derivatized crystals prepared by soaking were collected on films to 3.2-Å resolution. The Patterson rotation function, calculated with vectors in the 25–7Å range, demonstrates the presence of a local 4-fold axis parallel to the crystallographic *c* axis, and a local 2-fold axis in the *a*,*b* plane. The structure was solved to 5-Å resolution by the single isomorphous replacement (SIR) method, considering anomalous scattering contributions (Table 1). The electron density map (Fig. 1) was improved using solvent flattening[12,13] followed by 4-fold rotation averaging and phase combination[13]. The crystallographic 2-fold rotation axis along *a* relates the top and the bottom halves of the L_8S_8 molecule to each other and introduces a 36-Å shift between the two halves as the local 4-fold symmetry axes are 18 Å away from the twofold axis parallel to *a* (Fig. 4*a*); the point group thus reduces to C2. The L_4S_4 half of the molecule displays four protrusions (Fig. 1) and a central depression is observed in upper layers of the electron density map (Fig. 4).

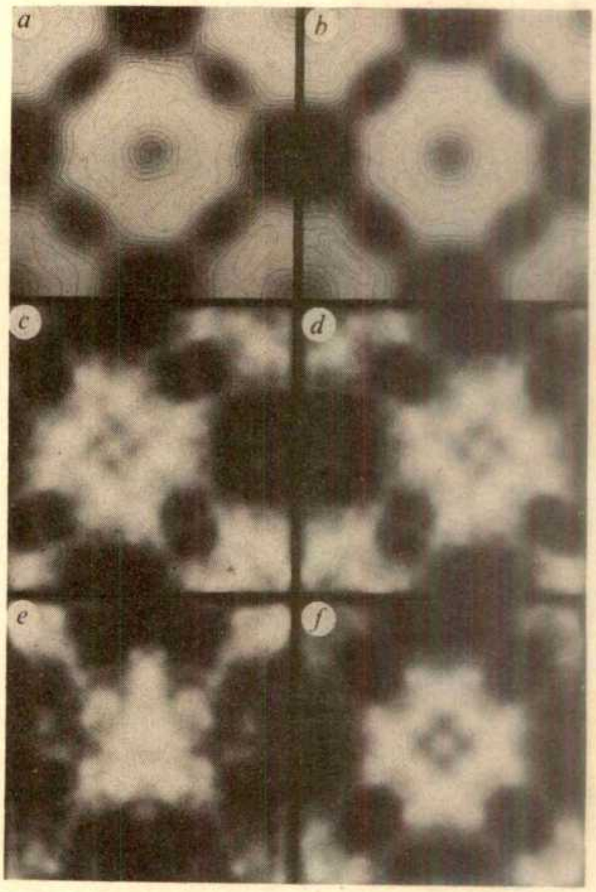

Fig. 3 Computer image analysis of electron micrographs (*a*, *b*) and comparison with X-ray electron density (*c*–*f*). *a*, Correlation averaging result with 17-Å resolution; *b*, the image in *a* with 4 mm symmetry imposed. *c*, *d*, Projections through the top and lower half molecules (each L_4S_4). *e*, Total projection (top and bottom) through the C2 molecule; *f*, total projection (top and bottom) through the molecule after removal of the 36-Å shift between half molecules. Full 4 mm symmetry is exhibited.

For electron microscopy, *A. eutrophus* H16 (DSM 428) RuBPCase was purified[2] using autotrophically grown[14], RuBPCase derepressed[15] cells. Purity of the fully active enzyme was greater than 98%, as determined by analytical SDS polyacrylamide gel electrophoresis[16]. The carboxylase activity was measured by the incorporation of $^{14}CO_2$ into acid-stable product at 30 °C[2]. Using a modification of a previously described method for the spinach RuBPCase[17], the enzyme was crystallized into a monolayer directly on the carbon support film used for electron microscopy. For crystallization the buffer was equivalent to that applied throughout the purification. However, it was supplemented with ammonium sulphate up to 7.77% saturation; it contained HCO_3^- and Mg^{2+} ions necessary to maintain RuBPCase activity.

The plane group of the 2D crystals in projection (Fig. 2) appears tetragonal (p4mm) with lattice dimension $a = 117 \pm 5$ Å. Diffraction maxima up to the fifth order (~23 Å) can be detected. The micrograph shown in Fig. 2 was analysed by correlation averaging[18] with the IMAGIC image processing system[19]. In this technique, the image of the 2D crystal is correlated with respect to a small reference crystal patch. The cross-correlation

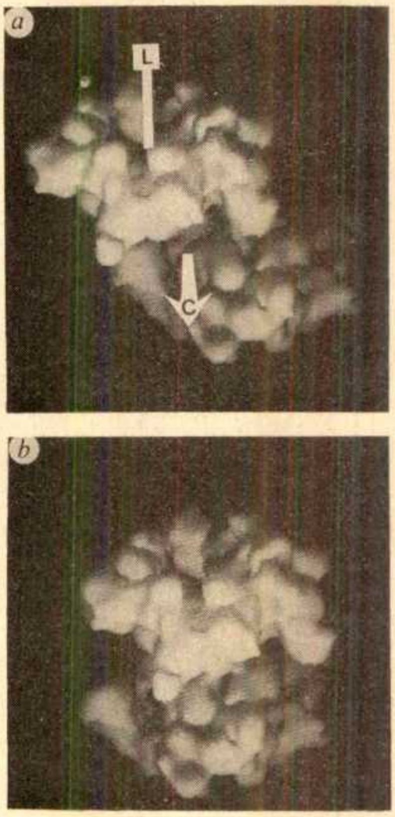

Fig. 4 Surface representation of the enzyme configuration derived from the 5-Å electron-density map of *Alcaligenes eutrophus* RuBPCase, where local 4-fold (L) and crystallographic 2-fold (C) symmetry elements are marked. *b*, Surface representation of the D4 model calculated from the X-ray electron density map after undoing the 36-Å shift between half molecules. In both *a* and *b*, the X-ray electron density map was low-pass filtered to 20-Å resolution to emphasize the quaternary structure. A surface representation algorithm was used[23,24].

map resulting from this operation contains peaks at the best match positions which also correspond to the positions of the individual unit cells. Using the coordinates of these peaks, the individual unit cells are extracted from the crystal and summed to give an image with an enhanced signal-to-noise ratio. Figure 3*a* shows the average of 900 aligned unit cells with a reproducible resolution of 17 Å as determined by Fourier ring correlation[18,20]. Because correlation averaging removes crystal distortions by translational alignment, the resolution of the final sum is better than could be achieved by simple Fourier peak filtering (17 Å compared with 23 Å)[18,19].

The average image (Fig. 3*a*) exhibits 4-fold symmetry even before 4mm symmetrization (Fig. 3*b*). The molecule has a central stain pit and is connected to its neighbours by eight 'arms'. The 4-fold symmetry indicates that the distribution of negative stain is almost uniform throughout the molecule, and is not restricted to only one half (L_4S_4). In contrast, molecules within 2D crystals rotationally shadowed with platinum-carbon show a strong handedness (data not shown). The electron micrographs analysed here of negatively stained specimens represent projections throughout the entire structure, as has been previously demonstrated for other molecules[21].

The lattice repeat of 117 ± 5 Å of the 2D crystal agrees well with the crystallographic data, where 112.4 Å is half of the crystal diagonal with $a = 158.8$ Å and $b = 159.6$ Å, suggesting that the lateral molecular packing in the 2D and 3D crystals is similar. X-ray density maps of the top and bottom halves of the molecule were blurred to 12 Å resolution and projected along the *c* axis (Fig. 3*c, d*) for comparison with electron microscope projections. Summing both halves gives a total projection through the structure (Fig. 3*e*) which only exhibits mirror symmetry, because the projection is perpendicular to the 2-fold axis of the C2 structure. When each half is shifted by 18 Å to make the local 4-fold axes coincide, the projection through the whole molecule becomes 4-fold symmetric (4 mm) (Fig. 3*f*) and corresponds closely with the electron microscope projections (Fig. 3*a, b*). We conclude that the point symmetry of RuBPCase complexed with the transition state analogue CABP in the $C222_1$ 3D crystal and of RuBPCase without CABP in the p4 2D crystal are essentially different (C2 and D4 respectively) (Fig. 4). Despite the introduction of the lateral shift between the half molecules (Fig. 4*a*), the nature of the lateral intermolecular contacts in both crystals remains almost identical.

Further crystallographic studies seek to derive a detailed molecular structure to higher (3.2-Å) resolution. However, even at the present resolution level (X-ray, 5 Å; EM, 17 Å), good agreement between results obtained independently using different approaches emphasizes the reliability of our data and leads to the conclusion that we have studied the molecule in two different structural states. In the $C222_1$ crystals, the 36-Å shift between the half molecules may have been induced by the presence of CABP. The alternative explanation, that the 3D crystal was composed of L_4S_4 half molecules with a non-crystallographic contact forming the octamers could be excluded: using low-speed sedimentation equilibrium centrifugation[4] the enzyme was shown not to decompose from its hexadecameric to an octameric state even at 25% ammonium sulphate saturation. Moreover, the idea that a lateral shift between half molecules may occur is supported by (1) independent X-ray studies with the tobacco enzyme[9] which indicate that the molecule can also assume D4 point group symmetry in 3D crystals obtained without HCO_3^-, Mg^{2+} and CABP; (2) biochemical studies on the spinach RuBPCase revealing different chromatographic and electrophoretic properties for the active and the CABP-treated enzyme[22]; and (3) preliminary electron microscope results performed on freeze-dried preparations of the enzyme complexed with CABP (data not shown). The relation between the two forms of RuBPCase and the active and inactive states of the enzyme remains to be investigated.

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The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor

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Alzheimer's disease[1] is characterized by a widespread functional disturbance of the human brain. Fibrillar amyloid proteins are deposited inside neurons as neurofibrillary tangles[2] and extracellularly as amyloid plaque cores[2] and in blood vessels[2]. The major protein subunit (A4) of the amyloid fibril of tangles, plaques and blood vessel deposits is an insoluble, highly aggregating small polypeptide of relative molecular mass 4,500[3-6]. The same polypeptide is also deposited in the brains of aged individuals with trisomy 21 (Down's syndrome)[3,5,6]. We have argued previously that the A4 protein is of neuronal origin and is the cleavage product of a larger precursor protein[4,6]. To identify this precursor, we have now isolated and sequenced an apparently full-length complementary DNA clone coding for the A4 polypeptide. The predicted precursor consists of 695 residues and contains features characteristic of glycosylated cell-surface receptors. This sequence, together with the localization of its gene on chromosome 21, suggests that the cerebral amyloid deposited in Alzheimer's disease and aged Down's syndrome is caused by aberrant catabolism of a cell-surface receptor.

We reasoned that the precursor of the A4 polypeptide may be produced in large amounts during the whole of life by cells of the human central nervous system. We therefore used brain tissue of a five-month-old fetus without trisomy 21 to obtain the messenger RNA needed to construct a cDNA library[7]. We used a probe designed from the N-terminal part of the A4 polypeptide to screen the library (details in Fig. 1 legend), and found several positive clones. The first one sequenced (clone 9-110) encoded the entire A4 subunit protein.

If we assume that translation starts at the first AUG, the protein would have 695 residues. It begins with a signal sequence (Figs 1 and 2) for transport through the endoplasmic reticulum membrane[8]. The signal sequence is followed by a region rich in cysteine. The last of 12 cysteines occurs at residue 187. Thus, the exact folding of these N-terminal regions may be stabilized by disulphide bridges. The next hundred residues are of peculiar composition. They include a stretch of seven uninterrupted threonine residues and are extremely rich in glutamic (28 residues) and aspartic (17 residues) acids, but contain only one lysine and two arginines. This domain would be able to bind large numbers of positive ions or polycations and may function in the electrical activity of nerve cells. The region from residue 290 to residue 597, the N-terminus of the amyloid A4 protein, contains two potential *N*-glycosylation sites at positions 467–469 and 496–498.

Like the anionic domain, the sequence of the amyloid A4 protein is also unique. We determined the complete amino-acid sequence of the A4 polypeptide isolated from both amyloid plaque cores and neurofibrillary tangles (for details see Fig. 2 legend). At most the amyloid subunit can be either 42 or 43 residues long and shows N-terminal heterogeneity as expected from previous work[3,4,6]. The 43-residue protein terminates at, and the 42-residue protein terminates just before, the first of the two threonine residues found in the potential transmembrane region (residues 625–648). The C-terminal cleavage of mature precursor during amyloidogenesis would thus have to occur within the membrane. Such a cleavage seems unlikely and the details of this process deserve further attention. The C-terminal end of the precursor is short (57 residues) compared to the cytoplasmic domain of known receptor proteins[9]. Following the putative membrane domain, there are three lysine residues (649–651) which probably interact with the phospholipid head groups in the membrane and are a characteristic feature for the junction between membrane and cytoplasmic domains of cell-surface receptors[9]. There is also a single sequence capable of asparagine-linked glycosylation (Asn-Pro-Thr). However, it is likely that this site is not glycosylated because of the presence of proline (Figs 1 and 2). The sequence and composition of the A4 precursor are not homologous to known proteins (see Fig. 1 legend) including the sequenced components of normal neurofilaments[10,11] or the PrP protein and gene isolated from the transmissible spongiform encephalopathies[12].

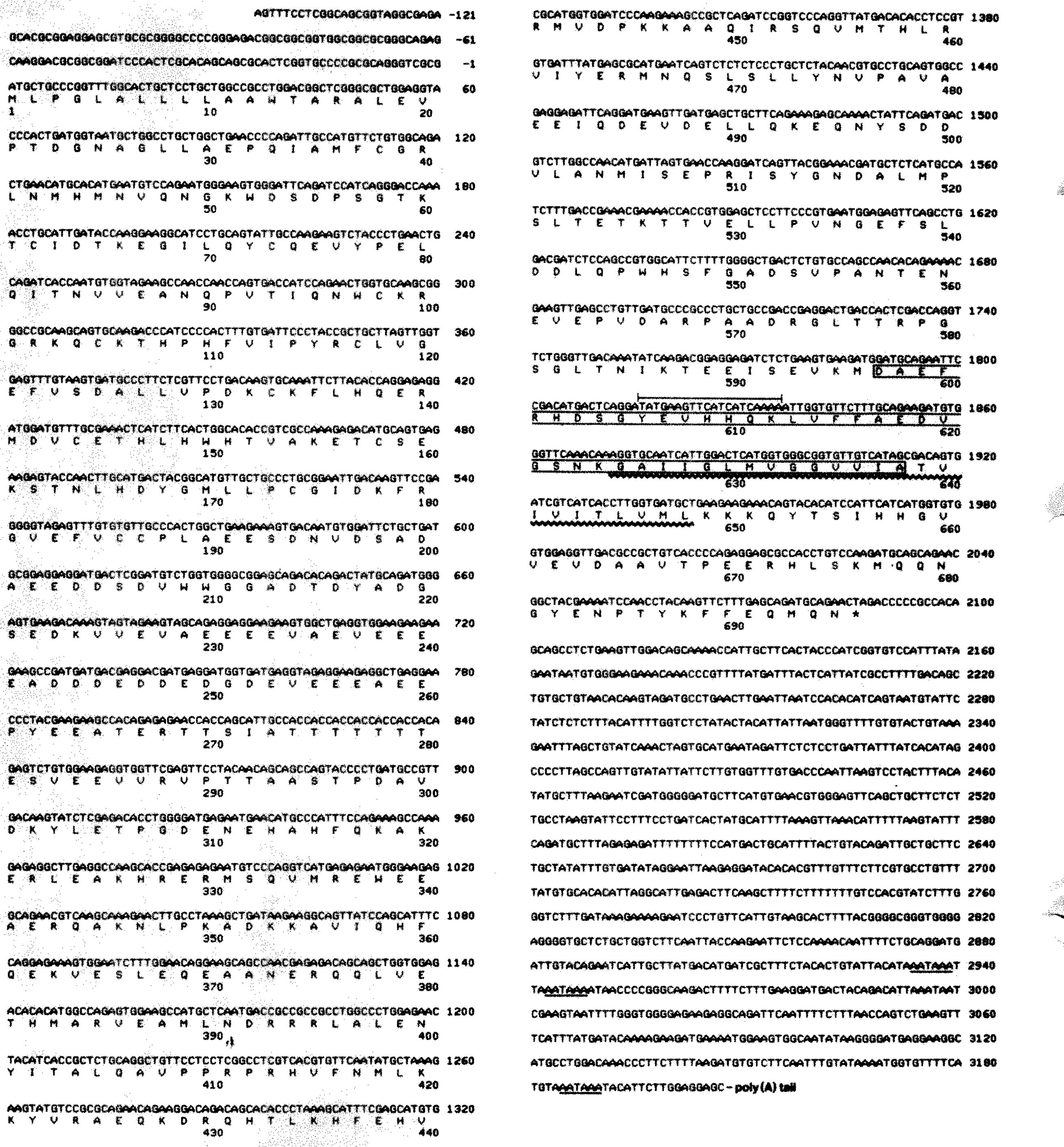
To determine the size of the mRNA encoding the A4 precursor we performed Northern blot hybridizations[13]. Total RNA was isolated from the same fetal cortex used for constructing the cDNA library. The RNA preparation was probed with the *Eco*RI fragment of the cloned A4 precursor cDNA (Fig. 3*a*). Two transcripts of 3.4 and 3.2 kilobases (kb) were detected. Both signals are in the size range of the cDNA insert described in Fig. 1. Similar results were obtained with mRNA from normal adult human cortex, and from cortex of sporadic Alzheimer's disease patients (data not shown).

Southern blot analyses[14] of DNA from 29 human × mouse cell hybrids[15] were used to localize human A4 precursor sequen-

Table 1 Chromosome location of human A4 precursor gene

Lane in Fig. 3*b*	Hybrid clone	Human chromosomes retained 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	Hybridization (8.8-kb band) in Fig. 3*b*
1	Human genome	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	+
2	RAG-SU 3-1-2-1			O		X	O	O														X		X		+
3	RAG RU 4-13	O		X		O	X	O			O				O			X		O		X	X	O	X	+
4	RAG 194-7	X		O	X	X			X		X		O							X		X		O		+
5	A9-SU 1-2		X	O			X							X	X				X			X				+
6	MS2 B82-1a-141		X	X	X			X	X			X		X		X	X	X	X	X		X	X	X	X	+
7	RAG ANLY 1	X		X	X	X	X						X		O				X			X				+
8	RAG 194-5-5			O	X	X	X	X			X		X	X	X		X				X	X	X	O		+
9	RAG GO-4			X	X			X	X					X	X	X			X				X	O		–
10	RAG PI 7-2	X		X		X	X	X	X	X		O	X		X	O			X				X	O		–
11	RAG 610-4-5-1	O		X	X	X	O						X	O	X	X			O		X		X	X		–
12	RAG mouse																									–

X, whole chromosome; O, chromosome fragment.



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                                  AGTTTCCTCGGCAGCGGTAGGCGAGA  -121

GCACGCGGAGGAGCGTGCGCGGGGCCCCGGGAGACGGCGGCGGTGGCGGCGCGGGCAGAG   -61

CAAGGACGCGGCGGATCCCACTCGCACAGCAGCGCACTCGGTGCCCCGCGCAGGGTCGCG    -1

ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGTA    60
M  L  P  G  L  A  L  L  L  L  A  A  W  T  A  R  A  L  E  V
1                          10                            20

CCCACTGATGGTAATGCTGGCCTGCTGGCTGAACCCCAGATTGCCATGTTCTGTGGCAGA   120
P  T  D  G  N  A  G  L  L  A  E  P  Q  I  A  M  F  C  G  R
                           30                            40

CTGAACATGCACATGAATGTCCAGAATGGGAAGTGGGATTCAGATCCATCAGGGACCAAA   180
L  N  M  H  M  N  V  Q  N  G  K  W  D  S  D  P  S  G  T  K
                           50                            60

ACCTGCATTGATACCAAGGAAGGCATCCTGCAGTATTGCCAAGAAGTCTACCCTGAACTG   240
T  C  I  D  T  K  E  G  I  L  Q  Y  C  Q  E  V  Y  P  E  L
                           70                            80

CAGATCACCAATGTGGTAGAAGCCAACCAACCAGTGACCATCCAGAACTGGTGCAAGCGG   300
Q  I  T  N  V  V  E  A  N  Q  P  V  T  I  Q  N  W  C  K  R
                           90                            100

GGCCGCAAGCAGTGCAAGACCCATCCCCACTTTGTGATTCCCTACCGCTGCTTAGTTGGT   360
G  R  K  Q  C  K  T  H  P  H  F  V  I  P  Y  R  C  L  V  G
                           110                           120

GAGTTTGTAAGTGATGCCCTTCTCGTTCCTGACAAGTGCAAATTCTTACACCAGGAGAGG   420
E  F  V  S  D  A  L  L  V  P  D  K  C  K  F  L  H  Q  E  R
                           130                           140

ATGGATGTTTGCGAAACTCATCTTCACTGGCACACCGTCGCCAAAGAGACATGCAGTGAG   480
M  D  V  C  E  T  H  L  H  W  H  T  V  A  K  E  T  C  S  E
                           150                           160

AAGAGTACCAACTTGCATGACTACGGCATGTTGCTGCCCTGCGGAATTGACAAGTTCCGA   540
K  S  T  N  L  H  D  Y  G  M  L  L  P  C  G  I  D  K  F  R
                           170                           180

GGGGTAGAGTTTGTGTGTTGCCCACTGGCTGAAGAAAGTGACAATGTGGATTCTGCTGAT   600
G  V  E  F  V  C  C  P  L  A  E  E  S  D  N  V  D  S  A  D
                           190                           200

GCGGAGGAGGATGACTCGGATGTCTGGTGGGGCGGAGCAGACACAGACTATGCAGATGGG   660
A  E  E  D  D  S  D  V  W  W  G  G  A  D  T  D  Y  A  D  G
                           210                           220

AGTGAAGACAAAGTAGTAGAAGTAGCAGAGGAGGAAGAAGTGGCTGAGGTGGAAGAAGAA   720
S  E  D  K  V  V  E  V  A  E  E  E  E  V  A  E  V  E  E  E
                           230                           240

GAAGCCGATGATGACGAGGACGATGAGGATGGTGATGAGGTAGAGGAAGAGGCTGAGGAA   780
E  A  D  D  D  E  D  D  E  D  G  D  E  V  E  E  E  A  E  E
                           250                           260

CCCTACGAAGAAGCCACAGAGAGAACCACCAGCATTGCCACCACCACCACCACCACCACA   840
P  Y  E  E  A  T  E  R  T  T  S  I  A  T  T  T  T  T  T  T
                           270                           280

GAGTCTGTGGAAGAGGTGGTTCGAGTTCCTACAACAGCAGCCAGTACCCCTGATGCCGTT   900
E  S  V  E  E  V  V  R  V  P  T  T  A  A  S  T  P  D  A  V
                           290                           300

GACAAGTATCTCGAGACACCTGGGGATGAGAATGAACATGCCCATTTCCAGAAAGCCAAA   960
D  K  Y  L  E  T  P  G  D  E  N  E  H  A  H  F  Q  K  A  K
                           310                           320

GAGAGGCTTGAGGCCAAGCACCGAGAGAGAATGTCCCAGGTCATGAGAGAATGGGAAGAG  1020
E  R  L  E  A  K  H  R  E  R  M  S  Q  V  M  R  E  W  E  E
                           330                           340

GCAGAACGTCAAGCAAAGAACTTGCCTAAAGCTGATAAGAAGGCAGTTATCCAGCATTTC  1080
A  E  R  Q  A  K  N  L  P  K  A  D  K  K  A  V  I  Q  H  F
                           350                           360

CAGGAGAAAGTGGAATCTTTGGAACAGGAAGCAGCCAACGAGAGACAGCAGCTGGTGGAG  1140
Q  E  K  V  E  S  L  E  Q  E  A  A  N  E  R  Q  Q  L  V  E
                           370                           380

ACACACATGGCCAGAGTGGAAGCCATGCTCAATGACCGCCGCCGCCTGGCCCTGGAGAAC  1200
T  H  M  A  R  V  E  A  M  L  N  D  R  R  R  L  A  L  E  N
                           390                           400

TACATCACCGCTCTGCAGGCTGTTCCTCCTCGGCCTCGTCACGTGTTCAATATGCTAAAG  1260
Y  I  T  A  L  Q  A  V  P  P  R  P  R  H  V  F  N  M  L  K
                           410                           420

AAGTATGTCCGCGCAGAACAGAAGGACAGACAGCACACCCTAAAGCATTTCGAGCATGTG  1320
K  Y  V  R  A  E  Q  K  D  R  Q  H  T  L  K  H  F  E  H  V
                           430                           440

CGCATGGTGGATCCCAAGAAAGCCGCTCAGATCCGGTCCCAGGTTATGACACACCTCCGT  1380
R  M  V  D  P  K  K  A  A  Q  I  R  S  Q  V  M  T  H  L  R
                           450                           460

GTGATTTATGAGCGCATGAATCAGTCTCTCTCCCTGCTCTACAACGTGCCTGCAGTGGCC  1440
V  I  Y  E  R  M  N  Q  S  L  S  L  L  Y  N  V  P  A  V  A
                           470                           480

GAGGAGATTCAGGATGAAGTTGATGAGCTGCTTCAGAAAGAGCAAAACTATTCAGATGAC  1500
E  E  I  Q  D  E  V  D  E  L  L  Q  K  E  Q  N  Y  S  D  D
                           490                           500

GTCTTGGCCAACATGATTAGTGAACCAAGGATCAGTTACGGAAACGATGCTCTCATGCCA  1560
V  L  A  N  M  I  S  E  P  R  I  S  Y  G  N  D  A  L  M  P
                           510                           520

TCTTTGACCGAAACGAAAACCACCGTGGAGCTCCTTCCCGTGAATGGAGAGTTCAGCCTG  1620
S  L  T  E  T  K  T  T  V  E  L  L  P  V  N  G  E  F  S  L
                           530                           540

GACGATCTCCAGCCGTGGCATTCTTTTGGGGCTGACTCTGTGCCAGCCAACACAGAAAAC  1680
D  D  L  Q  P  W  H  S  F  G  A  D  S  V  P  A  N  T  E  N
                           550                           560

GAAGTTGAGCCTGTTGATGCCCGCCCTGCTGCCGACCGAGGACTGACCACTCGACCAGGT  1740
E  V  E  P  V  D  A  R  P  A  A  D  R  G  L  T  T  R  P  G
                           570                           580

TCTGGGTTGACAAATATCAAGACGGAGGAGATCTCTGAAGTGAAGATGGATGCAGAATTC  1800
S  G  L  T  N  I  K  T  E  E  I  S  E  V  K  M  D  A  E  F
                           590                           600

CGACATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTG  1860
R  H  D  S  G  Y  E  V  H  H  Q  K  L  V  F  F  A  E  D  V
                           610                           620

GGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGACAGTG  1920
G  S  N  K  G  A  I  I  G  L  M  V  G  G  V  V  I  A  T  V
                           630                           640

ATCGTCATCACCTTGGTGATGCTGAAGAAGAAACAGTACACATCCATTCATCATGGTGTG  1980
I  V  I  T  L  V  M  L  K  K  K  Q  Y  T  S  I  H  H  G  V
                           650                           660

GTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCACCTGTCCAAGATGCAGCAGAAC  2040
V  E  V  D  A  A  V  T  P  E  E  R  H  L  S  K  M  Q  Q  N
                           670                           680

GGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAACTAGACCCCCGCCACA  2100
G  Y  E  N  P  T  Y  K  F  F  E  Q  M  Q  N  *
                           690

GCAGCCTCTGAAGTTGGACAGCAAAACCATTGCTTCACTACCCATCGGTGTCCATTTATA  2160

GAATAATGTGGGAAGAAACAAACCCGTTTTATGATTTACTCATTATCGCCTTTTGACAGC  2220

TGTGCTGTAACACAAGTAGATGCCTGAACTTGAATTAATCCACACATCAGTAATGTATTC  2280

TATCTCTCTTTACATTTTGGTCTCTATACTACATTATTAATGGGTTTTGTGTACTGTAAA  2340

GAATTTAGCTGTATCAAACTAGTGCATGAATAGATTCTCTCCTGATTATTTATCACATAG  2400

CCCCTTAGCCAGTTGTATATTATTCTTGTGGTTTGTGACCCAATTAAGTCCTACTTTACA  2460

TATGCTTTAAGAATCGATGGGGGATGCTTCATGTGAACGTGGGAGTTCAGCTGCTTCTCT  2520

TGCCTAAGTATTCCTTTCCTGATCACTATGCATTTTAAAGTTAAACATTTTTAAGTATTT  2580

CAGATGCTTTAGAGAGATTTTTTTTCCATGACTGCATTTTACTGTACAGATTGCTGCTTC  2640

TGCTATATTTGTGATATAGGAATTAAGAGGATACACACGTTTGTTTCTTCGTGCCTGTTT  2700

TATGTGCACACATTAGGCATTGAGACTTCAAGCTTTTCTTTTTTTGTCCACGTATCTTTG  2760

GGTCTTTGATAAAGAAAAGAATCCCTGTTCATTGTAAGCACTTTTACGGGGCGGGTGGGG  2820

AGGGGTGCTCTGCTGGTCTTCAATTACCAAGAATTCTCCAAAACAATTTTCTGCAGGATG  2880

ATTGTACAGAATCATTGCTTATGACATGATCGCTTTCTACACTGTATTACATAAATAAAT  2940

TAAATAAAATAACCCCGGGCAAGACTTTTCTTTGAAGGATGACTACAGACATTAAATAAT  3000

CGAAGTAATTTTGGGTGGGGAGAAGAGGCAGATTCAATTTTCTTTAACCAGTCTGAAGTT  3060

TCATTTATGATACAAAAGAAGATGAAAATGGAAGTGGCAATATAAGGGGATGAGGAAGGC  3120

ATGCCTGGACAAACCCTTCTTTTAAGATGTGTCTTCAATTTGTATAAAATGGTGTTTTCA  3180

TGTAAATAAATACATTCTTGGAGGAGC - poly(A) tail
```

Fig. 1 Nucleotide sequence and predicted amino-acid sequence of a cDNA clone encoding the precursor of the amyloid A4 protein of Alzheimer's disease. Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first base of the initiation codon AUG. The untranslated sequence directly following the poly(G) tail is indicated by negative numbers. The sequence shows a 695-residue open reading frame. The deduced amino-acid sequence is numbered, the amino-terminal methionine included. The amino-acid sequence of the A4 polypeptide (includes data from refs 3 and 4) is boxed. Zigzag underline, a probable membrane-spanning sequence of the A4 polypeptide. The synthetic oligonucleotide mixture used as probe is indicated as a line above the corresponding cDNA. Polyadenylation signals are underlined. Nucleotide 3207 is followed by a poly(dA) tail linked to the vector DNA. A computer search for homology was unsuccessful using computer programs of the University of Wisconsin Genetics Computer Group (UWGCG). The entire cDNA insert includes 3,353 bp and *Eco*RI sites at bp positions 1,795 and 2,851. The amino-acid composition of the 695-residue A4 precursor is A57, C12, D47, E85, F17, G31, H25, I23, K38, L52, M21, N28, P31, Q33, R33, S30, T45, V62, W8, Y17 resulting a calculated M_r of 78,644.45.

Methods. Total RNA was extracted[24] from cortex of the brain of a 5-month-old aborted human fetus. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography[25]. A cDNA library was constructed by the method of Okayama and Berg[7] with 3 μg poly(A)$^+$ RNA and 1.5 μg vector-primer DNA. The cDNA was transformed into *Escherichia coli* HB101. Transformants (10^5) were screened[26] with a probe consisting of a mixture of 64 20-mers, $5'\mathrm{TT^{T}_{C}TG^{A}_{G}TG^{A}_{G}TGCAC^{T}_{C}T^{CA}_{GG}TA}3'$, deduced from residues 10–16 of the A4 polypeptide. Of the nine positive clones obtained, one (clone 9–110) was sequenced by the dideoxy chain termination method[27] on both strands with the help of synthetic primers. The primers were synthesized on a DNA-synthesizer (Applied Biosytems)[28]. For sequence comparison, UWGCG programs Wordsearch and Gap were applied to the data libraries of EMBL and NBR7.

a

A Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-
1 5 10

Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe
11 15 20

Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala
21 25 30

Ile-Xxx-Xxx-Leu-Met-Val
31 35 36

B Gly-Ala-Ile-Xxx-Xxx-Leu-Met-Val
29 36

C Val-Gly-Gly-Val-Val-Ile-Ala
36 42

D Val-Gly-Gly-Val-Val-Ile-Ala-Thr
36 43

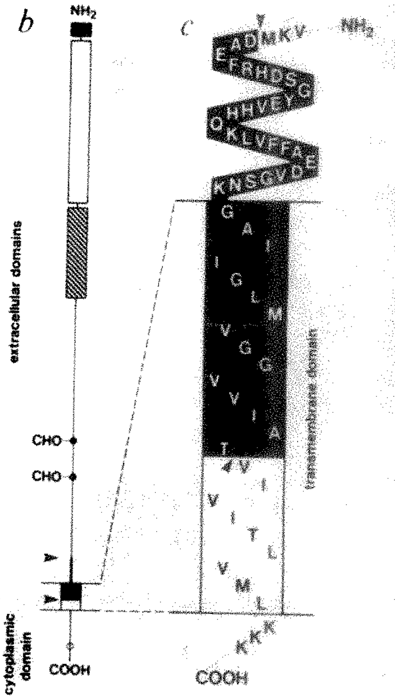

Fig. 2 Model of A4 precursor as a cell-surface glycoprotein. *a*, Amino-acid sequence of amyloid plaque core-A4 polypeptide. Amyloid from plaque cores was purified from human post-mortem brain homogenates[3,6]. The amino-terminal sequence shown (A) was obtained for amyloid plaque core-A4 polypeptides. To determine the internal and the C-terminal portion of the A4 polypeptide, the corresponding trypsin digestion (B) and cyanogen bromide cleavage products (C, D) were sequenced. However, we were unable to assign residues 32 and 33 unambiguously. Amyloid plaque core preparations from different Alzheimer brains suggest that the amyloid protein may consist variably of at most 42 or 43 residues (C, D). *b*, Proposed domain structure: black box, signal sequence[8], open box, cysteine-rich region; hatched box, highly negatively charged domain (45% Asp and Glu residues); filled circles, *N*-glycosylation sites. The A4 amyloid is shown in black between the two arrowheads and reaches into the (boxed) hydrophobic transmembrane segment. The putative cytoplasmic domain includes the sequence Asn–Pro–Thr (open circle). *c*, The full-length A4 amyloid protein (42–43 residues) as deposited in the brain of patients with Alzheimer's disease (shaded region), includes 28 residues of the extracellular domain and 14–15 residues of the transmembrane domain of the A4 precursor. The group of three Lys residues following the 24 hydrophobic amino acids of the putative transmembrane domain are typical for the junction between membrane and cytoplasmic domains of cell surface receptors[9].

Methods. The amyloid plaque cores were extracted with formic acid as described[3,4] and the formic acid-soluble protein was applied to the gas-phase protein sequencer (Model 470 A, Applied Biosystems) described by Hewick *et al.*[29]. The amino-acid derivatives released were determined by reverse-phase HPLC in on-line configuration using 40% of the sample (Model 120A, Applied Biosystems). Four different amyloid plaque core preparations were sequenced; typically, 400 pmol of total N-terminal derivatives was obtained from 900 pmol of protein (estimated by quantitative amino-acid analysis). For the tryptic digest in *b*, 10 pmol of sequence was obtained from the estimated 5 nmol of acetylated protein; 20 pmol (*c*) and 50 pmol (*d*) of sequence was obtained for the cyanogen bromide cleavages from an estimated 2 nmol of acetylated protein (details to be published elsewhere).

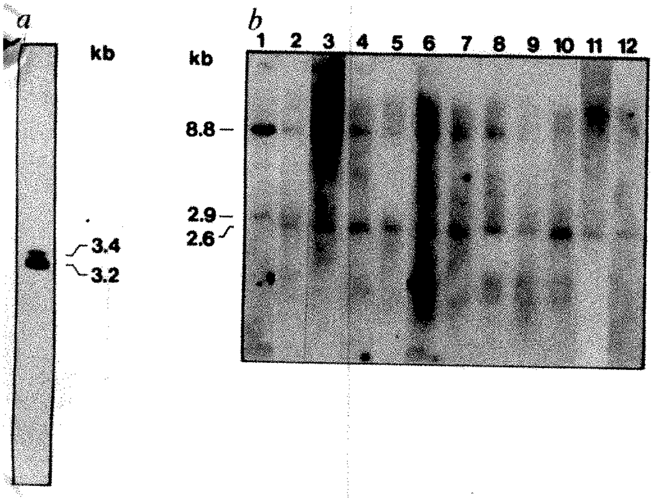

Fig. 3 Hybridization analyses. *a*, Identification of A4 precursor mRNA by Northern blot analysis[13]. RNA was isolated from human embryonic cortex as in Fig. 1. RNA (25 μg) was heated for 15 min at 65 °C in the presence of 50% formamide, subjected to electrophoresis on a 1.6% agarose gel containing 6 M urea/25 mM sodium citrate, *p*H 3.5[30] and transferred to Zetabind membrane (AMF Cuno). Blots were hybridized at 65 °C with nick-translated[31] 1,056-bp *Eco*RI fragment of clone 9-110 (bp positions 1,795–2,851, Fig. 1) in 6 × SSC, 50 mM sodium phosphate *p*H 6.5, 5 × Denhardt's, 0.5% SDS, 1 mM EDTA, $100\ \mu g\ ml^{-1}$ denatured calf thymus DNA. The filters were washed to a final stringency of 0.2 × SSC, 0.1% SDS at 65 °C. Autoradiography was for 24 h at −70 °C using intensifying screens. An RNA ladder (BRL) was used as size marker. *b*, Chromosome mapping of A4 precursor locus. The 1,056-bp cDNA *Eco*RI fragment of clone 9-110 (Fig. 1) was used for Southern blot analysis[14] of *Eco*RI-digested human × mouse RAG or mouse A9 hybrid cell line DNAs. DNA from the somatic cell hybrids was purified from isolated nuclei as described in detail elsewhere[15]. The *Eco*RI-digested DNA (~10 μg) from each of 29 hybrid cell samples was electrophoresed through a 0.8% agarose gel and blotted onto nylon (Pall). Results from 10 of the 29 hybrids analysed are presented here and in Table 1. Hybridization and washing were as in *a*. Lane 1, human genomic DNA; lanes 2–8, hybrid cell lines containing human chromosome 21; lanes 9–11, hybrid cell lines missing human chromosome 21; lane 12, mouse RAG genomic DNA. The chromosome distribution is given in Table 1.

ces to chromosome 21. The ^{32}P-labelled *Eco*RI fragment of the human A4 precursor cDNA was hybridized to Southern filters of *Eco*RI-digested DNA from control tissue and hybrid cell lines. Two human-specific fragments of 8.8 and 2.9 kb and a mouse-specific fragment of 2.6 kb were observed (Fig. 3*b*). The 8.8 kb fragment was used to score for the presence of the human A4 precursor gene in the DNA from the hybrid cell lines. Chromosome 21 was the only human chromosome that showed perfect concordance with human A4 precursor sequences (Fig. 3*b*, lanes 2–8, and Table 1). Every other human chromosome could be ruled out by at least two discordant hybrids.

It may be significant that chromosome 21 is the chromosome that is duplicated in Down's syndrome. We have evidence that the A4 gene is close to the part of the chromosome that is duplicated (unpublished results). There could be a more complex interaction between other chromosome 21 gene products and the biosynthesis and processing of the A4 precursor in Down's syndrome, rather than a simple gene-dosage effect.

The transmissible spongiform encephalopathies, scrapie, CJD and kuru[16] are progressive degenerative disorders of the nervous sytem sharing many clinical and pathological features with Alzheimer's disease. In these unusual infectious diseases, an abnormally post-translationally modified protein (PrP)[12] accumulates in the form of amyloid structures in the brain[17-20]. However, the corresponding gene for the PrP protein precursor has been localized to chromosome 20[21]. Nevertheless, we and Oesch *et al.*[12] show that the two proteins share many similarities, including features characteristic of glycosylated membrane receptors. It is possible that the putative membrane-spanning domains of both proteins share an amyloid-forming or amyloid-inducing potential.

The availability of a complete A4 precursor cDNA now permits a systematic study of the normal and abnormal biology of this amyloidogenic protein in Down's syndrome, familial and sporadic Alzheimer's disease and related disorders. During the preparation of this manuscript we have learnt that two other groups have isolated cDNA clones coding for A4[22,23].

We thank Robert G. Rohwer and Caroline Hilbich for comments, Klaus Olek for a gift of Okayama-Berg primer, Thomas Herget for RNA, and Brigitte Kisters and Pauline Klein for help with the computers. This work was supported by grants from the Deutsche Forschungsgemeinschaft through SFB 74 A1, A2 and GR 373, and the National Health and Medical Research Council of Australia. The sequence data in this publication have been submitted to the EMBL/GenBank Libraries under the accession number Y00264.

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Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

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Myths and mad March hares

HOLLEY and Greenwood[1] claim that boxing in hares is the female's attempt to prevent mating, and that 'madness' is not a feature of March. The behaviour they describe as boxing I call rebuff[2]. An unreceptive female lunges at a male that comes too close, usually from the semi-crouched threat position. Boxing between males establishes dominance[3]. In Germany the characteristic erect posture of two males feinting with the fore paws, 'Distanzgefecht' or real boxing in contrast to 'Brustkampf' the usual female rebuff, has been photographed in marked individuals[4,5]. Dr E. Schneider (in litt., 2/9/84) recorded boxing at least 40 times, all male-male in the 16 bouts with sexes identified. In 17 rebuffs, 14 involved females. That no male-male boxing was recorded by Holley seems curious; perhaps his population was so isolated the males all knew their ranking.

Testosterone levels in English hares rise dramatically from February to April, so interaction between males is likely to peak in March, as do daylight sightings of adult hares in Norfolk[6]. In New Zealand where turf remains short throughout the year, hare sightings peak in September (the equivalent of March). Hence I do not agree that male 'mad March hares' are a myth, or that female hares do the boxing.

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Sperm sharing in *Biomphalaria* snails

SINCE 1968, as an inference from observations published later[1], I hypothesized that a simultaneous hermaphrodite snail with a planorbid-type reproductive system could transfer sperm from a donor to a recipient snail. Such hypothesis, tested in numerous crossing experiments with *Biomphalaria glabrata*, proved untenable. The report by Monteiro, Almeida and Dias[2] led me to repeat my former (unpublished, of course) experiments, this time strictly following the procedure described by those authors. I had previously performed unsuccessfully similar experiments at the same laboratory of the University of Brasilia, and with the same strain of *B. glabrata* successfully handled by these authors.

Concerning *B. tenagophila*, in which transference of sperm from donor to recipient through an intermediate snail is asserted to have occurred in 20 out of 70 experiments (about 28%), I carried out tests with 47 trios, using the same strains that were used by the mentioned authors: NB-JO-JO (14), NB-JO-BH (9), NB-BH-JO (10), JP-JO-JO (7), JP-BH-JO (7). These trios correspond to the most successful combinations mentioned by Monteiro *et al.*, since transference of sperm is said to have occurred in 18 experiments (about 38%). In my trials, however, not even a single candidate recipient produced any embryo resulting from fertilization by donor's sperm during a period of 100 days from the 'intermediate-recipient' pairing. The only explanation that occurs to me for the alleged transference of sperm is that there was confusion of albino 'intermediate' and albino 'recipient' during the mating period, when similar snails were paired and separated every day.

Another disputable statement made by Monteiro *et al.* is that 'crossbreeding between *B. tenagophila* and *B. occidentalis* is common in the laboratory'. In my description of the latter as a new species[3], I emphasized that it is indistinguishable from *B. tenagophila* by the shell characteristics, and showed that the two species differ morphologically in some features of the genital system and are separated by absolute reproductive isolation. The latter characteristic was now confirmed, when I failed to interbreed *B. occidentalis* from Sena Madureira (SM) and *B. tenagophila* to form the trios SM-BH-BH, SM-JO-BH and SM-JO-JO, for a complete lack of hybridization between SM-BH (10 couples) and SM-JO (10 couples). My explanation of the successful results mentioned by Monteiro and colleagues is that in their experiments *B. tenagophila* was mistaken for *B. occidentalis.*

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MONTEIRO *et al.*[1] describe sperm sharing in simultaneously hermaphroditic *Biomphalaria* snails, a strategy inconsistent with most models of mating systems. We suggest that this represents 'cheating' in a sperm-trading mating system, such as that found in the opisthobranch, *Navanax inermis*[2-4], and predicted for pulmonate gastropods[4].

Recent theories of mating systems are based on Bateman's principle[5], which states that fecundity of females is limited by the energy available for egg production, and that of males by access to females or eggs. Therefore, simultaneous hermaphrodites should mate 'not so much to gain sperm to fertilize eggs as to give sperm away (to gain access to another's eggs)'[6]. That is, they should trade eggs, as has been described for *Hypoplectrus*, a serranid fish[7]. In contrast, sperm sharing represents 'a strategy enabling the snail to receive much sperm from its partners, when it is acting as a functional female, while transferring relatively few spermatozoa of its own'[1]. This suggests that *Biomphalaria* are trading sperm rather than eggs.

In a sperm-trading mating system, individuals mate as males not so much to fertilize eggs as to receive sperm to fertilize their own eggs (as in *Navanax*[2-4]). Comparison of the mating systems of *Hypoplectrus* and *Navanax* suggests that the risk of gamete loss (rather than the energy invested in gametes) determines the 'preferred' sexual role. Simultaneous hermaphrodites should trade sperm, rather than eggs, wherever sperm are at a greater risk of loss before fertilization[3]. In *Biomphalaria*, eggs are assured of fertilization because females can self-fertilize if necessary[8]. However, copulating as a male does not guarantee access to eggs since *Biomphalaria* store foreign sperm and digest the excess[9,10]. To act as a female should therefore be the preferred role in *Biomphalaria.*

Sperm sharing represents a way of reciprocating with a partner without producing any (or as many) sperm of one's own, as these may be digested rather than used in fertilization. It is a way of 'cheating' as a male. Sperm sharing will not harm the female unless the female received its own sperm back from its partner. We expect that mechanisms to prevent this should exist since cross-fertilization is preferred in *Biomphalaria*[11].

Monteiro *et al.*[1] suggested that sperm sharing might represent an evolutionarily stable strategy related to a bias towards female function. However, *Hypoplectrus*, which trades eggs, has sex allocation biased toward female function[12] and Bateman's principle[13] cannot explain why an animal should transfer foreign sperm to a partner.

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MONTEIRO *ET AL.* REPLY—We agree with Leonard and Lukowiak that *Biomphalaria* snails must have reproductive strategies similar to those found in *Navanax*[1] and the finding of sperm trading in these snails will certainly help to explain why sperm sharing should occur[2]. However, egg trading (that is, ovule trading) in *Hypoplectrus* only becomes an evolutionary stable strategy[3,4] when accompanied by sex allocation biased towards female function. Fischer argues that this is due to decrease in fitness gain in relation to further increments in resources expended in male function[5], but did not explain why this should happen. Accordingly, we believe sperm trading also needs this type of sex allocation to be an evolutionary stable strategy[5].

Bias towards female function in both *Hypoplectrus* and *Biomphalaria*, and possibly in *Navanax*, may be explained by lower competition between eggs than between spermatozoa[6]. *Hypoplectrus* have highly dispersed planktonic eggs[3] with virtually no competition with eggs from the same parent, whereas spermatozoa compete among each other for the limited number of ovules available in each spawn (ovule parcelling[5]). In *Biomphalaria* and *Navanax* spermatozoa must compete for the limited number of ovules available in recipient snail, while young offspring of same mother readily disperse locally with no apparent competition for resources among them. In *Biomphalaria*, spermatozoa might also compete to fertilize ovules from the same parent in the case of selfing. Hence, sex allocation biased towards female function probably plays a role in the maintenance of sperm sharing in *Biomphalaria*[2]. Given the capacity of these snails to store sperm for up to five months[2] and to digest it[7,8], it is possible that much of the sperm traded serves a nutritive function.

Thus, sperm sharing could be an evolutionary stable strategy in the presence of female function when there is bias towards female function which would enable the snail to trade sperm with other individuals while minimizing the risk of losing its own sperm.

Dr Paraense reports his unsuccessful trials on repeating sperm transference from donor to recipient through an intermediate snail. A mixture of recipient and intermediary snails could not have occurred in our experiment because recipients were tagged with a red bead glued to the shell. This detail was not mentioned in the paper for two reasons: one, limitation on the space; the other is the irrelevance of the detail, because in planning to perform an experiment with two albino snails, the implication is that I could tell which was which. Furthermore, it is obvious that if intermediate and recipient snails were both laying wild-type hybrid eggs that had been mixed, the results still show that the recipient snail was laying wild-type eggs. As a matter of fact, Figure 2 shows that intermediary as well as recipient snails had laid wild-type hybrid eggs concomitantly, for at least one hundred successive days.

That we have mixed *B. tenagophila* and *B. occidentalis* is possible as nobody can identify the two species based on shell characters alone. As stated, our colleague Mara L. F. Dias and I are studying the occurrence of crosses between these two species, identifying them on the basis of reproductive organs as recommended in the paper where *B. occidentalis* was first described[3]. We only admit to dealing with *B. tenagophila* instead of *B. occidentalis* if the diagnostic characters for *B. occidentalis* are not actually diagnostic. In this case those characters must be reevaluated to identify properly the species *B. occidentalis*[3]. After all snails with *B. occidentalis* characters do crossbreed with snails with *B. tenagophila* characters in our laboratory.

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The measurement of gross planktonic production

THERE are conflicting reports in the literature concerning the reliability of the ^{14}C method, with indirect estimates of primary productivity differing from those provided by the ^{14}C technique by factors of anywhere from 2 to 100 (ref. 1). The implications of such errors are far-reaching. Platt *et al.*[2] have pointed out that in some instances the reports have been based on comparison of methods of fundamentally different time scales and the comparison is therefore suspect, if not invalid. From paired observation of *in vitro* oxygen- and ^{14}C-derived measurements of production, we have concluded that we had no evidence for errors unique to the ^{14}C technique in the measurement of gross primary production (GPP). We felt that this point had to be established with an oligotrophic population before undertaking direct comparisons of *in vitro* and *in situ* rates, which can be done with oxygen but not ^{14}C. This would provide a chain of logic to enable us to determine whether there are in fact large errors associated with the ^{14}C technique. No claim was made as to the accuracy of the *in vitro* techniques, although we did note that there was circumstantial evidence for their accuracy.

Smith *et al.*[3] have argued that our approach did not have the necessary rigour to allow us to draw our conclusion. On the basis of a hypothetical food chain they argue that what they regard to be positive errors in the ^{14}C technique could be compensated for by an overestimate of photosynthetic oxygen production, resulting from a rundown of respiration in the dark, the latter predicted from an empirical model. Even if the situation they envisage did exist in the samples we studied, the errors that the uncertainties would introduce (5-10%) are small in comparison with those we wished to consider. In the text we inadvertently used the ambiguous phrase "of any size". However, we presume it was evident from our introduction that our paper was concerned with errors of 2-100-fold (that is, the matter of the current debate over oceanic productivity measurements). Smith *et al.* also model unpublished data from a quite different location to illustrate how time-dependent changes might give rise to larger errors. The experimental protocol we used (in which we measured respiration rate after 0, 12, 24 and, in one instance, 36 hours of darkness and dissolved and particulate organic ^{14}C measurement at the end of the light and the following dark period) was adopted to give warning of errors of this type. Thus we contend that our approach was adequate for the question we set out to answer and that the arguments of Smith *et al.* are not grounds for rejecting our conclusion.

Finally we must make it clear that it was not our wish to argue that the ^{14}C technique gave an exact measurement of GPP. It has been recognized for over two decades that the compartmentation of tracer introduces uncertainties in the interpretation of ^{14}C measurements. However, it is evident from our original paper that we share the views of Smith *et al.* and others (for instance ref. 4) that these types of errors, present in incubations *in vitro*, are not the prime cause of the large purported underestimates of GPP.

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SMITH *ET AL.* REPLY—The debate over the true level of ocenaic primary productivity has tended to focus on possible shortcomings of the ^{14}C technique[1]. A recurrent suggestion is that ^{14}C results are biased by the activity of heterotrophic organisms in the sample. The potential respiration of fixed tracer carbon by heterotrophic, as well as autotrophic, organisms makes it difficult to specify what level of production[2] is measured by ^{14}C and complicates comparisons against other means of estimating production. It seems less well recognized that methods other than ^{14}C can be similarly biased. We discussed[3] the particular case of comparisons of O_2 with ^{14}C, using data from the literature for two different oceanic areas. We concluded that both ^{14}C and O_2 could underestimate GPP by factors ranging from near zero to at least twofold, depending on the trophic make-up and cycling rate of the communities, yet display excellent mutual agreement. The key to mutual agreement in the face of widely varying absolute bias and high community respiration rates was the metabolic dominance of microheterotrophs.

Williams and Marra wish to emphasize that their comparative measurements[4] did at least refute the existence of errors much greater than twofold, unique to the ^{14}C technique, in the estimation of GPP in their samples. We would, and did, agree. Their original paper[4] was not so clear on the scale of error in question. The abstract pointed specifically to the question of whether ^{14}C measures gross or net primary productivity, and it was concluded (p. 50) that "the technique measures gross rather than net production". It appeared to be their contention that the measurements could discriminate gross from net primary productivity (in the sense of ref. 2, cited in ref. 4) but it is now explicitly agreed that they could not.

The experimental design of Williams *et al.*[4] was admirably thorough and provided a number of tests for the larger bias predicted by our model for other oceanic areas. In fact, it was the availability of such tests in their data that allowed us to fit[3] a model to the results and derive evidence for a metabolically dominant microheterotroph population. The model then allowed us to interpret observations on other oceanic areas to show that the trophodynamics of microplankton, and the magnitude of bias in production rates, was likely to differ considerably from that observed by Williams *et al.*[4]. An important point was that such differences would not be revealed by a simple comparison that treated the O_2 method as an absolute standard rather than as an operational production estimate. This conclusion was intended to be cautionary rather than to be critical of the particular findings of Williams *et al.*[4].

Our model[3] was extremely simple and of mainly heuristic value, but we should mention that Williams and Marra describe it incorrectly. The model entertains only negative errors, or underestimates, in measurements of GPP by both ^{14}C and O_2. An erroneously low value for respiration in the O_2 dark bottle is predicted from the model, not from an empirical relationship, and such an error can cause only an underestimate of GPP, not an overestimate as Williams and Marra suggest. Loss of fixed tracer can cause only an underestimate of GPP by ^{14}C. Both mechanisms are recognized as sources of error in production estimates[1,3] but only more recently have their quantitative implications been modelled[3,5].

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Hercynian orogeny in the Pyrenees was not a rifting event

THE Pyrenees were a segment of Hercynian crust that underwent rifting during the Mesozoic followed by compression during Late Cretaceous to Cenozoic time[1-3]. From a regional study of Hercynian metamorphism in a North Pyrenean massif it was recently proposed[4] that 'the Hercynian orogeny in the Pyrenees was a rifting event . . . that affected a continuously subsiding Palaeozoic basin'. We believe that a rifting hypothesis for the Hercynian evolution of the Pyrenees is unlikely for the following reasons.

(1) The Pyrenees have suffered a strong pre-Permian and post-Carboniferous shortening resulting in polyphase deformation[5-9]. This deformation is manifested by recumbent folding[10-16], nappe emplacement[17-19] and subsequent steep axial-plane folding associated with strong slaty cleavage; Devonian and Carboniferous series are affected by these events[20-22]. A late kinematic high-grade Hercynian metamorphism also developed in both the North Pyrenean zone and the Axial zone, where it affects the Devonian sequence[7,15].

(2) The Hercynian terrains of the Pyrenees represent only a small piece of a much larger Hercynian belt which extends across all of western Europe. The Hercynian is now considered to have been a typical collisional orogeny resulting from the closure of at least two oceanic domains[23]. Taking into account the late Hercynian strike-slip faulting and the Mesozoic-Cenozoic displacement of Iberia relative to Europe along the North Pyrenean fault[3], we conclude that the North Pyrenean massifs and the Axial zone of the Pyrenees are both external parts of the Hercynian belt. Thus the 'schematic setting' only related to rifting, proposed by Wickham and Oxburgh, is not in agreement with existing structural data in the Pyrenees, nor with the regional geology of western Europe.

Of course, a strong rifting did occur in the Pyrenees in association with strike-slip motion, but it began only in the early Cretaceous[24] and was accompanied by locally high-grade metamorphism[25], well known in the Mesozoic rocks. The Hercynian basement itself was affected by this Pyrenean event, as demonstrated by ^{40}Ar-^{39}Ar dates in certain Pyrenean massifs, such as the St Barthélemy massif (H. Maluski, personal communication).

Contrary to the proposed rifting model[4], the Hercynian metamorphism in the Pyrenees accompanies or just follows a strong pre-Permian, post-Carboniferous shortening observed everywhere in the Pyrenean basement. Thus it must be related to a thickening and not to a thinning of the crust.

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WICKHAM AND OXBURGH reply—Matte and Mattauer raise two objections to our interpretation of the Hercynian orogeny in the Pyrenees as a rifting event[1,2,3], the first relating to the Hercynian deformation style, the second to the regional tectonic interpretation of the entire Hercynian belt of Western Europe.

In the matter of Hercynian deformation style, we have no serious disagreement concerning many of the features described by Matte and Mattauer. We do not, however, believe that it is possible unambiguously to deduce the regional tectonic setting from such features. To the best of our knowledge no-one has a clear idea of the strain pattern expected in the middle crust during a strike-slip rifting event. We could certainly imagine heterogeneous deformation in such a setting that gave rise to features similar to those observed.

The second point raised by Matte and Mattauer is that rifting in the Pyrenees is not consistent with regional interpretations of the tectonic setting of the Hercynian of western Europe[4]. The Hercynian belt covers a very large area, and metamorphism and plutonism extended from the Devonian to the Permian[5,6]. We do not believe that it is reasonable to ascribe a single tectonic mechanism to all of these events. Studies of recent orogenic belts have revealed considerable complexity in the spatial and temporal juxtaposition of different tectonic styles and thermal regimes[7-11]. For example, the North Island of New Zealand contains an active rift zone characterized by high heat flow, hydrothermal systems and silicic magmatism[8,10]. The crust of this region has many geological and geophysical similarities to the Hercynian crust of the Pyrenees[1-3]. Yet this zone is located only 300 km west of an active subduction zone within which low-temperature, high-pressure metamorphism and compressional deformation are occurring.

It is unlikely that large-scale crustal thickening occurred in the Pyrenees during the Hercynian orogeny because late Carboniferous uplift was not great. Post-orogenic Upper Carboniferous and Lower Permian red beds and volcanics commonly rest unconformably on marine Carboniferous and Devonian sediments, but never directly overlie high-grade basement rocks[2,12]. This is in marked contrast with the large-scale uplift and deep erosion levels observed in modern collision belts, such as the Alps[13] or the Himalayas[14]. Furthermore, there is a correlation between stratigraphic age, intensity of deformation and grade of metamorphism experienced by the Hercynian basement rocks in the Pyrenees. Upper Palaeozoic rocks have in general been only weakly deformed and metamorphosed, whereas Lower Palaeozoic metasediments experienced strong ductile deformation and amphibolite-facies metamorphism. This observation precludes the transport of near-surface material to deep structural levels in the crust, which is a common feature of overthrust terrains and collision belts.

The timing of the Hercynian metamorphism is fundamental to any tectonic interpretation. Age determinations on synmetamorphic granites in the Pyrenees have yielded ages of 335 ± 15 Myr (whole-rock Rb-Sr on the "granite profond" at Canigou[15]) and 338 ± 5 Myr (ion-probe U-Pb on leucogranite zircon from the Trois Seigneurs massif; I. S. Williams, S. M. Wickham and W. Compston, in preparation). These Visean to Namurian ages are contemporary with Upper Carboniferous marine sedimentation documented throughout the entire Pyrenean region[16]. Metamorphism was contemporary with marine sedimentation at the surface. This situation is radically different from that expected in collision belts, where uplift precedes metamorphism by tens of millions of years[13,17] but is entirely compatible with a rift setting[1-3,18].

The comments of Matte and Mattauer do not address two of the most remarkable features of the Hercynian Pyrenees, which we believe to be diagnostic of a rift setting. These are the exceptional thermal regimes which existed locally during metamorphism[3,12,19], and the deep hydrothermal systems which resulted in infiltration of the high-grade Palaeozoic metasediments by surface-derived marine fluids[20,21]. Neither of these features is compatible with the known thermal and mechanical effects of crustal thickening[9], but they are very similar to the observed geological and geophysical features of continental rift zones[1,10,18,22-24]. A rift setting is further supported by the total absence of any high-pressure metamorphic rocks in the Pyrenees, by the lack of ophiolitic or oceanic rock types, and by the contemporaneity of marine sedimentation at the surface and metamorphism at depth. We re-state our conclusion that Hercynian metamorphism in the Pyrenees occurred within localized rift zones, accompanied by intrusion of mantle-derived mafic material at depth. Rifting affected a deep, laterally extensive Palaeozoic sedimentary basin and promoted the low-pressure regional metamorphism, crustal anatexis and deep hydrothermal systems that characterize Hercynian metamorphic terrains in the Pyrenees.

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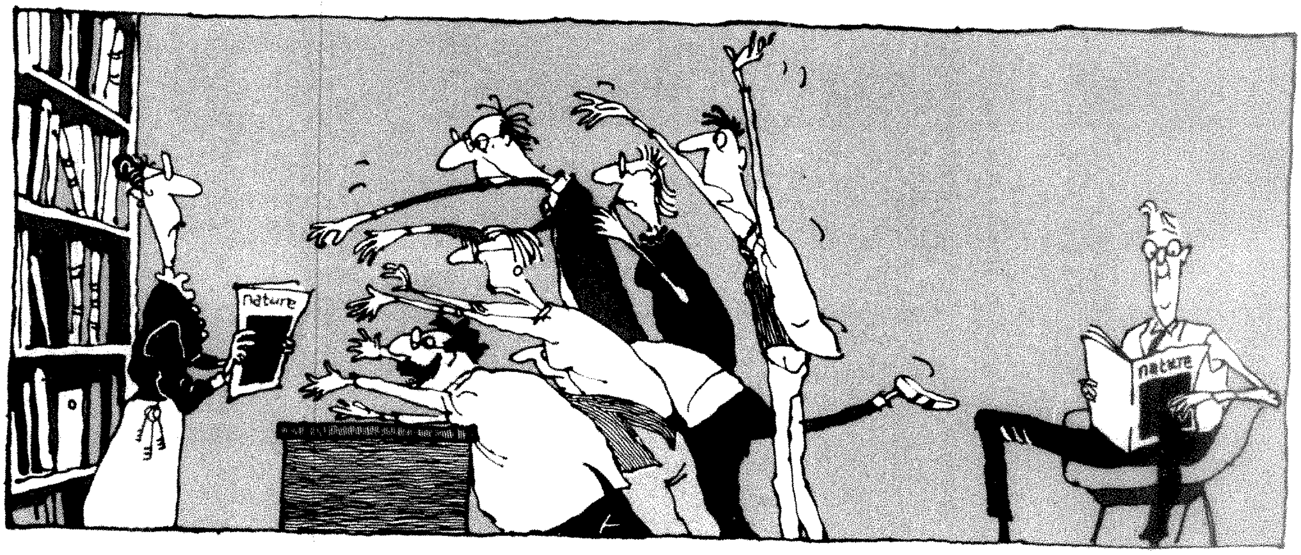

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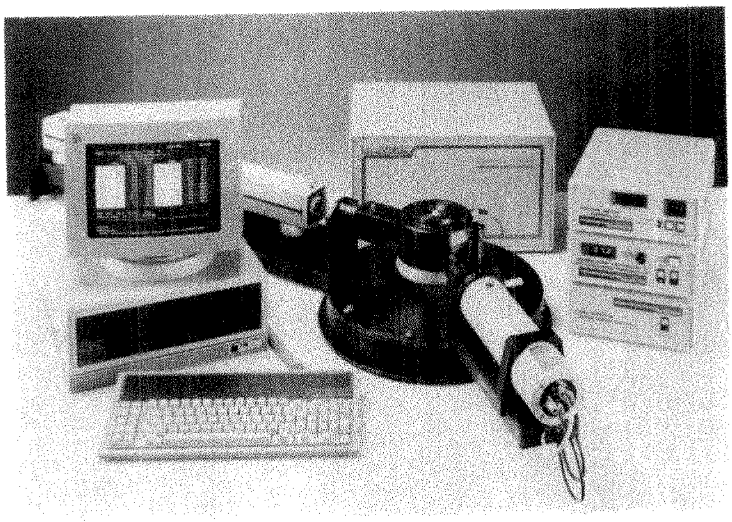

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Flow cytometry in biomedical science

James V. Watson

Lasers have significant applications in biology, as recent advances in flow cytometry demonstrate.

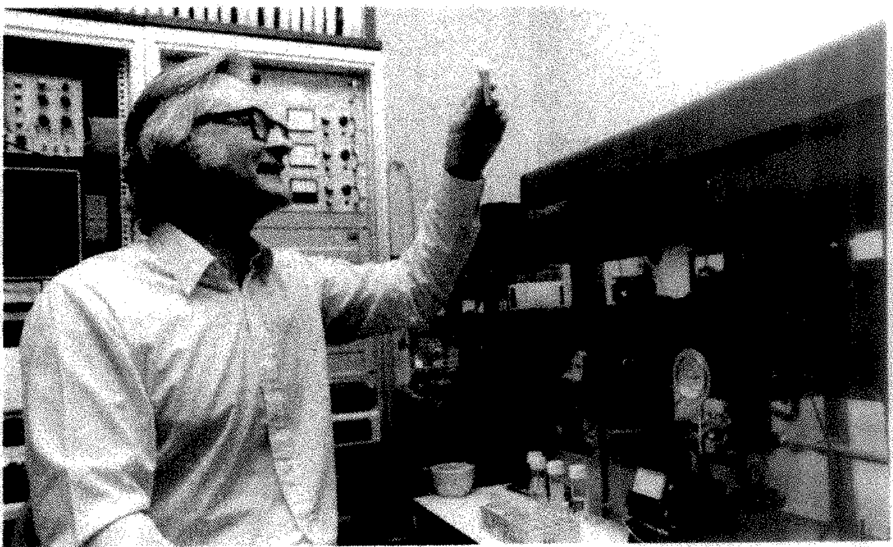
FLOW cytometry (FCM) is being used increasingly in all branches of biological and medical science due to its quantitative capacity, versatility, sensitivity, speed, statistical precision, and ability to identify cell subsets in, and sort them from, heterogeneous populations. Quantitation of FCM relies upon both fluorescence and light scatter measurements from individual cells, and the power of the technique derives from the ability to make these multiple measurements of each cell simultaneously, at very rapid rates. Typically, up to 5,000 cells can be analysed per second and sorting rates of 3,000 to 4,000 cells per second with greater than 90 per cent purity can be achieved.

Streams of cells

A single cell suspension is streamed coaxially through a flow chamber so that individual cells pass single file through the focus of a high intensity excitation light source. Lasers are frequently used because the laser light beam is very stable and easily focused to small volumes, and the high light flux excites sufficient fluorescence from individual cells to be measured in the few microseconds taken to traverse the focal volume. Each flash of scattered light emitted from a cell, or fluorescence from cells containing suitably stained molecules, is picked up by a light collection system and filtered sequentially by dichroic mirrors. These reflect specific wavelengths into photomultipliers (PMTs) which are additionally 'guarded' by band pass filters. The quantity of light incident on each PMT, responding within its given colour band, is then proportionally converted to an electrical signal. This is amplified, digitized, stored transiently in computer memory and thence stored on disk for subsequent display and analysis.

Many commercial machines are now equipped with four detectors and two lasers, for two- or three-colour fluorescence work. However, some multi-laser research and development instruments have up to eight detectors[1] and one such instrument has the ability to collect data on 32 light scatter detectors simultaneously[2]. Data processing and display procedures have been developed which handle up to 8-dimensional fully cross-correlated data in a single pass through the data set, using microcomputers with only 28K addressable memory[1].

Cell sorters originate from developments in ink jet writing[3]. These instruments deflect electrostatically charged droplets, each of which contains a single cell with given fluorescence and light scatter characteristics, into collection vessels for subsequent identification or biological manipulation.


James V. Watson seated before the Cambridge flow cytometer, the prototype for most current flow cytometers.

Staining techniques

The applications of this technology are legion and are increasing as the sensitivity increases and as new probes are developed. A detection limit equivalent to 150 molecules of free fluorescein per cell has been achieved[4], and with innovations currently under development this could be further improved by a factor of five to ten. Fluorescent dyes and staining techniques fall into three categories: those in which the ligand itself is fluorescent and is accumulated within the cell, those that require interaction of the ligand with a cellular constituent to release the fluorophore or enhance its emission, and fluorochrome-coupled antibodies.

Nucleic acid dyes have been used extensively in FCM and include DNA- or nucleic acid-specific ligands and polyanion dyes such as acridine orange, for both monovariate and bivariate cell cycle analysis. Acridine orange has the unusual property of giving rise to green fluorescence from double-stranded nucleic acids and red fluorescence from single strands, and selective denaturation of double-stranded RNA gives a simultaneous quantitation of both nucleic acids[5]. This dye has also been used in a cervical cancer prescreening programme in conjunction with slit scanning to give nuclear/cytoplasmic ratios. Blind trials of the technique exhibited a false negative rate of 0.18 per cent and about a 10 per cent false positive rate[6].

The bis-benzimidazoles bind in the minor groove of the DNA molecule, and have interesting properties because their emission spectrum when bound to DNA is dependent on the DNA:dye ratio and on the *p*H of the microenvironment. At low dye:DNA ratios the fluorescence emission is short wavelength (violet)-biassed but at higher ratios the emission is long wavelength (green)-biassed[7]. These differences can be measured by using two or more detectors responding to different regions of the emission spectrum[8]. This phenomenon has enabled different subsets to be identified within heterogeneous populations, enabling the identification of cells resistant to cytotoxic agents, which excrete dye molecules by the same mechanisms they use to excrete the drug. Hence, cells resistant to the cytotoxic agent exhibit a violet-biassed Hoescht 33342 emission spectrum, while cells that succumb to the cytotoxins display a longer wavelength-biassed emission spectrum.

Nuclei can be extracted from archival human biopsies for DNA ploidy studies using a number of fluorochromes, including DAPI and ethidium bromide[9]. Such studies can reveal aneuploid components, yielding information on which to base future prognoses[10]. These techniques have been extended to include the simultaneous assay of DNA and nuclear-associated oncogene-encoded proteins[11] which may be clinically useful for cervical cancer prescreening[12]. The simultaneous determination of total versus newly replicated DNA can also be assayed with similar types of techniques using anti-BrdU antibodies[13] or biotinylated nucleotides[14]. DNA fluorochromes are also used for chromosome analysis with both single[15] and dual laser[16] systems, not only for automated karyotyping, but also for constructing cloned gene libraries from individually sorted chromosomes[17].

Kinetic measurements of both cytoplasmic[18] and plasma membrane[19] enzymes have been developed to give K_m and V_{max} for reactions in populations of viable intact cells, using fluorogenic substrates based on fluorescein and methyl-umbelliferones. These methods have now been extended to determine the level of reaction inhibition induced by carbamylating cytotoxic agents. This is an indirect method for assaying the transport of the agent across the external membrane, and for determining its cytotoxic efficacy once it enters the cell[20].

Fluorophore-coupled monoclonal antibodies have been used in conjunction with FCM in a bewildering number of studies in both cell biology and medicine. Perhaps the most widely studied are the haemopoetic and related systems. Automated leucocyte typing in leukaemias, immunosuppressed transplant patients and lymphomas are now routine in many hospitals. The rapid progress in elucidating details of bone marrow lineages[21] over the last five years has been due to the development of suitable monoclonal antibodies, and to the refinement of cell sorting flow cytometry techniques. □

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The latest in lasers

This week features new developments in lasers and their accessories, plus a review of laser-based instruments for biological applications.

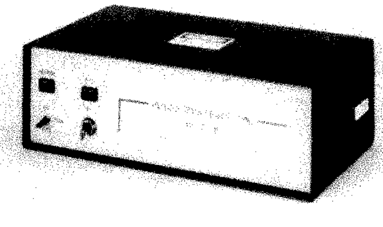
COHERENT has a wide range of **small frame ion lasers** for a variety of applications (Reader Service No. 100). The Innova 90 has a metal ceramic tube design, and is now available with a 6 W specification, completing the existing Innova 90 range which includes 3, 4 and 5 W argon systems and a krypton version. The £28,600 (UK) Innova 90 has a series regulated power supply, a remote control module, and a sealed intracavity space, and has now been expanded to incorporate a longer plasma tube, and a super invar resonator. The Innova 70 series is designed for high performance in the 2 to 4 W range at low cost, priced at around £12,500 (UK). Other features include light and current control, aperture wheel, compensated wavelength selector and all-line mirror mount. The Innova 70 comes as a package with a dye laser and power meter.

The new Microyag-1 **diode-pumped Nd:YAG laser** from Photon Control Ltd combines the best of both laser types (Reader Service No. 101). The low-divergence beam of the Microyag-1 has an output wavelength of either 1.06 or 1.3 µm, and the Microyag-1 has continuous wave, gain switched, pulsed and optional Q-switched operating modes. Costing £8,400–£14,400 (UK) Photon Control's new laser has applications in fibre optic sensor research, coherent communications, injection locking and inspection and testing.

Combines the best of two laser types.

Spectra-Physics has extended its line of **dye lasers**. The Model 355 ion-mountable dye laser combines with the Model 2020, 2025, or 168 argon ion lasers to form a compact dye laser system (Reader Service No. 102). The £10,000 (UK) Model 355 uses a choice of two dyes, rhodamine 6G or kiton red, with maxima of 595 and 640 nm, respectively, for a tuning range of more than 40 nm for each dye. Specified power exceeds 750 mW at the peak of each dye. The two-mirrors of the Model 355 produce high conversion efficiency and high spectral purity, according to Spectra-Physics, and the birefringent filter is set in the cavity at Brewster's angle for minimal loss.

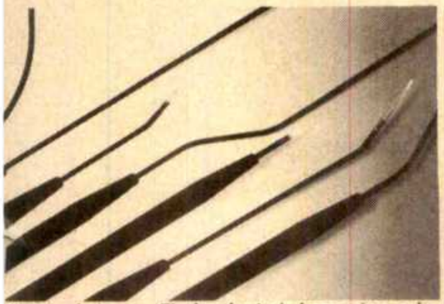
Pulse Systems, Inc. makes **CO_2 lasers** with full-width, half-maximum pulse widths of approximately 75 µs (Reader Service No. 103). The pulse shape can be varied from 10 ns to >200 µs using other main-pulse discharge circuits and/or by adding Q-switching and cavity dumping to the laser cavity. The $3,498–$19,000 (US) unit is complete, with all power supplies, pulse electronics and internal-trigger system included. Pulse System's lasers can operate without a helium supply, using a 50-50 mixture of CO_2 and air, and are therefore insensitive to O_2.



A CO_2 laser that doesn't require a helium supply and is insensitive to O_2.

The **pulsed Nd:YAG lasers** available from Laser Photonics are geared toward research applications (Reader Service No. 104). The modular design of Laser Photonics' products allow it to accommodate most customized laser needs upon request. The unique design of Laser Photonics' transmitter module employs a folded-beam resonator, a configuration that eliminates problems caused by misalignment of mirrors due to shock, vibration and thermal stress. Closed cycle cooling means no outside water supply is needed for operation, and a simmered flash lamp supply achieves pulse-to-pulse stability. Laser Photonics' line of Nd:YAG lasers range in price from $15,000 to $25,000 (US).

Lasers are making a name for themselves in the surgical theatre, and Surgical Laser Technologies, Inc. is providing **contact lasers** to lead the way (Reader Service

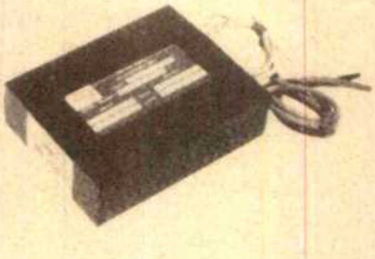


Surgical Laser Technologies' laser tips take accurate aim in laser surgery.

No. 105). Its $65,000 (US) contact laser combines the attributes of CO_2 lasers for cutting, YAG lasers for coagulating, and argon lasers for subsurface coagulation into one portable unit. Surgeons change the sapphire tip to enable them to perform these operations with little preparation. The low power operation of the contact lasers means no uncontrollable laser energy backscatter that can damage healthy tissue around the incision.

Accessories for lasers

Power Technology, Inc. makes **power supplies** to keep lasers running (Reader Service No. 106). The Series L23 laser power supplies offered by Power Technology are compact, solid state, regulated power supplies designed for use with helium neon laser tubes. Many shapes and sizes are available to fit a large variety of packages. Switching mode regulation provides high efficiency, reducing the need for a heat sink, thus increasing the life of the laser tube seals and other heat sensitive components. The $50–100 (US) L23 series is suitable for virtually all 12V battery powered applications, according to Power Technology, and also for many a.c. line applications where 12 to 15V d.c. is available from a bulk power supply. Also for helium neon lasers, the L92 series is a current regulated, switching mode, dual input voltage power supply for operation from a.c. input voltages of 100–130V or 200–260V. The $60–110 (US) L92 is fully potted in black fibreglass casing for protection from dust and moisture.



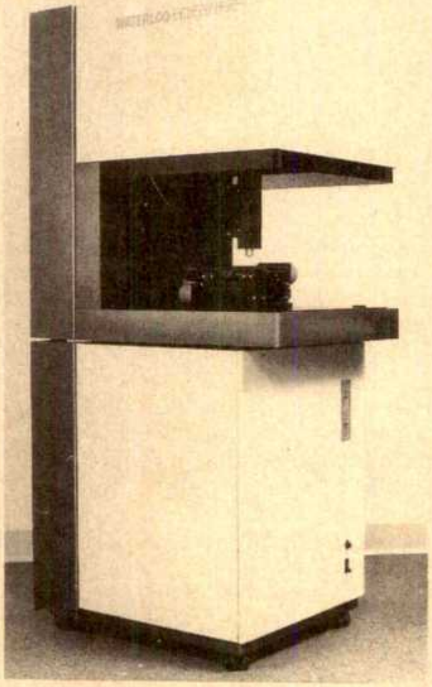
The power supply for helium neon lasers.

If **monitoring actual power level** during operation of high energy infrared lasers is a concern, Oriel Scientific Ltd offers the LIS-4000 Infrared Integrating Sphere (Reader Service No. 107). The 4-inch diameter water-cooled sphere has a gold coating, optimized for diffuse reflectance at the CO_2 laser wavelength, and can be used in conjunction with almost any infrared detector that mounts at a port on the integrating sphere. For monitoring during operation, the LIS-4000 can be mounted at the back mirror of the laser cavity. Oriel's £1,976 (UK) Infrared Integrating Sphere can also be supplied with a coating optimized at other infrared laser lines as well.

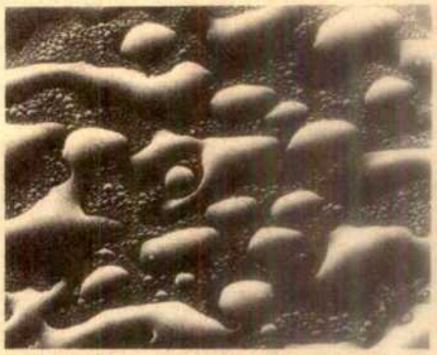
For real-time, high-resolution **images of infrared laser beams**, Oriel Scientific Ltd distributes Optical Engineering's water-cooled thermal image plates (Reader Service No. 108). According to Oriel, the water-cooled design allows continuous viewing of laser beams of up to 500 W. The fast response time permits viewing of rapidly changing laser mode patterns when adjusting resonator mirrors. The 5 × 5 inch plates come in four sensitivity ranges contained on two plates, and cost £236 (UK).

Laser-based instruments

The Waterloo Scientific WSI-100 **scanning laser microscope** uses a red HeNe laser beam focused to a diffraction-limited spot for optical beam-induced current measurements in semiconductors, solar cell and photovoltaic device testing, and automated testing of thin film devices and arrays (Reader Service No. 109). With the WSI-100, all functions are computer-controlled, as the specimen is moved in a raster scan on precision translation stages under a fixed laser beam. The resulting image is displayed on a high-resolution colour monitor, in false colour, gray scale, or Y-deflection line images (with or without hidden line eliminator). Image enhancement and analysis software is also included in the WSI-100 scanning laser microscope's $90,000 (US) price.



Uses a laser for high resolution microscopy.



At first glance appearing like water beading up on a waxed surface, this scanning electron micrograph actually reveals the result of piercing a silicon wafer with a laser. The laser beam drilled a hole by heating and almost instantly vapourizing the silicon, that subsequently condensed and resolidified on the surface of the wafer as tiny drops. This photo is one of 50 photomicrographs, photomacrographs, and electron microscopic images comprising the exhibit "MicroScapes: The Hidden Art of High Technology", on display at the US National Academy of Sciences until 12 March. Produced and developed by AT&T to display advanced developments in microelectronics and lightwave communications, "MicroScapes" represents some of the current processes used in the production of advanced communications systems. The exhibit includes micrographs of metal crystals, cross sections of preforms from which optic fibres called lightguide are drawn, and close-ups of microprocessor chips that reveal the complex configuration of circuits. Noteworthy among the collection is a scanning tunnelling micrograph of atoms on a silicon surface, the first glimpse of an atom gained via the Nobel-prize winning technology. The exhibition is on a three-year tour of the United States under the auspices of the Association of Science-Technology Centers in Washington, D.C., and will constantly be updated during its run — a reflection of the rapid pace in which new developments are being made in telecommunications.

From Wyatt Technology Corp. comes a **high performance laser chromatography system** for gel permeation chromatography (GPC) (Reader Service No. 110). The $18,500(US) DAWN GPC Model F multiangle laserlight scattering detector allows near real time determination of both absolute molecular weights and molecular sizes without calibration of the GPC column. Its low-volume flow cell allows low- and high-angle scattering measurements to be made instantly. The DAWN GPC contains four optical fibre-coupled photomultipliers placed at angular locations specified by the user, as well as 14 high-gain hybrid photodiode detectors spanning an angular range from about 10° to 160°. A 5 mW polarized HeNe laser is used as the light source, although tunable argon–ion and helium–cadmium lasers of 10–20 mW power options can be provided for increased sensitivity. The DAWN system may be connected to a standard concentration-sensitive detector, using its associated GPC software package written for IBM XT/AT computers. The software collects data continuously from the DAWN and the concentration detector for the duration of the chromatographic run, and calculates and graphs solvent baseline determinations as well as peak widths, heights and delay times.

Spectrolab Ltd combines a pulsed laser, a micro-zoom optic and an emission spectrometer to come up with its Laser Emission Microprobe, an instrument that succeeds in obtaining **atomic emission analysis** of microscopic-sized samples (Reader Service No. 111). Spectrolab's $35,000 (US) Laser Emission Microprobe uses a pulse of light from a laser, focussed onto an area of the sample no more than a few micrometres in diameter. The laser beam vapourizes the illuminated area, and the vapourized material triggers an electric arc between two electrodes, causing an emission of radiation that characterizes the sample. Spectolab claims the technique is so sensitive that a complete atomic analysis may be performed on samples only 20 μm in cross-section in just a few nanoseconds.

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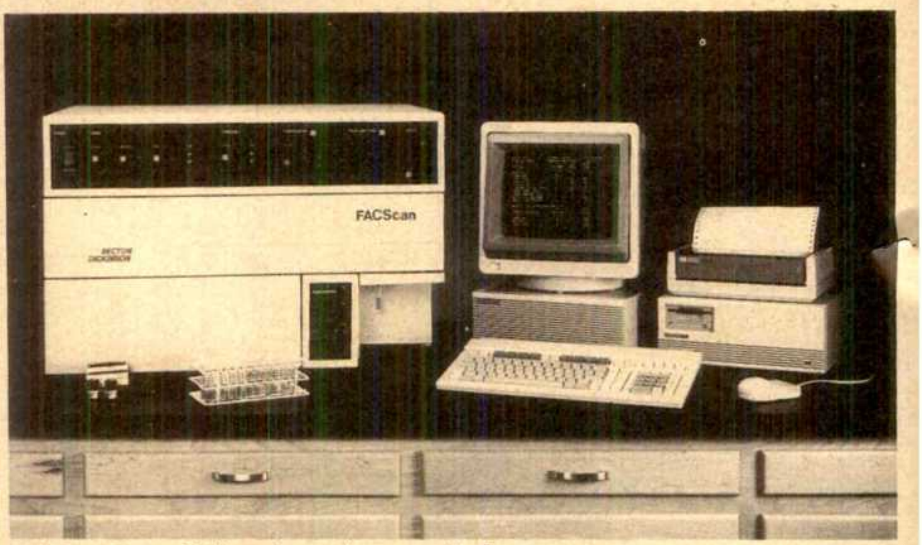
Reader Service No.47

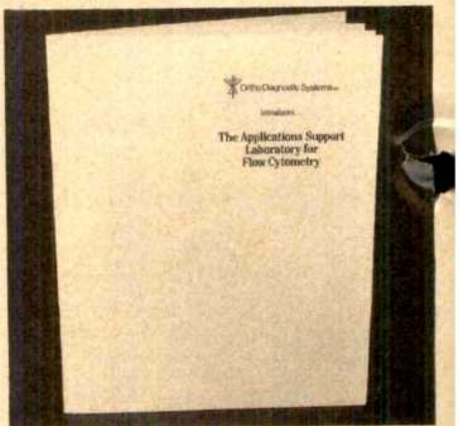
Malvern takes care of **particle sizing** needs with its MasterSizer, a laser-based particle size analyser for powders, sprays and emulsions (Reader Service No. 112). Using Mie light scattering analysis and the Fraunhofer diffraction method, the MasterSizer makes high-resolution measurements of powders down to the submicron range, according to Malvern. The MasterSizer accommodates analysis of particles in two size ranges: 0.1–80 μm and 1.2–600 μm. A third range, 0.5–170 μm, is also available. Each range is further subdivided into 64 size classes to maximize resolution. The MasterSizer has automatic calibration and detector alignment, and all operations can be automated by computer control, if required.

FACScan, the five-parameter **flow cytometer** developed by Becton Dickinson, fits right on the benchtop and needs no external supplies of water or compressed air (Reader Service No. 113). It is equipped with a 15 mW air-cooled argon-ion laser and three fluorescence channels (FITC, PE, and red fluorescence), plus forward scatter and side scatter. The $74,900 (US) FACScan comes with a Consort 30 computer workstation complete with monochrome monitor, allowing automated monitoring of sample flow and laser power, and analysis using menu-driven applications software for lymphocyte phenotyping, immune monitoring, DNA analysis, reticulocyte counting and platelet studies. Accessories for the FACScan include the FACS AutoMATE for sample introduction, and colour graphics.



FACScan: Becton Dickinson's complete system for flow cytometry.

To back up its line of flow cytometers, Ortho Diagnostic Systems is offering enrollment in its free **flow cytometry applications support** laboratory (Reader Service No. 114). Under the applications support programme, enrollees will have access to the laboratory's telephone consultation service for questions concerning flow cytometry, reagents or applications. Participants will also receive a complete bound set of Ortho Diagnostic Systems' *Protocols for Flow Cytometry*, a set of 15 step-by-step applications for flow cytometry in DNA analysis, cell viability testing, and differential cell analysis. ☐


Ortho Diagnostic Systems Inc.

The Applications Support
Laboratory for
Flow Cytometry

Outlines the details of Ortho Diagnostic's application support for flow cytometry programme.

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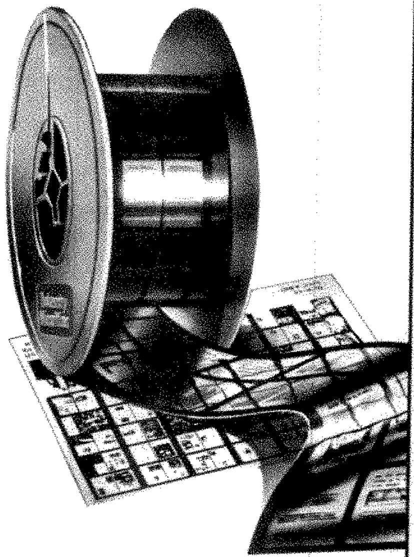

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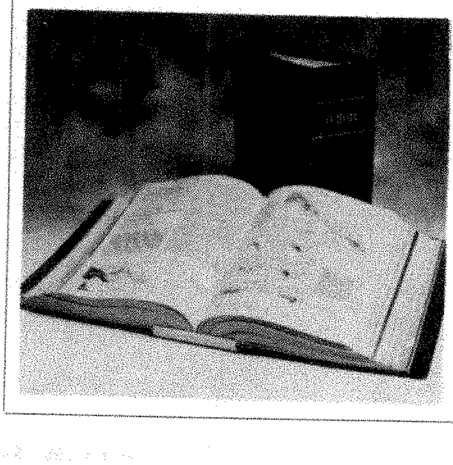

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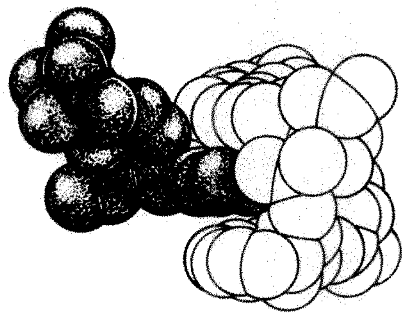

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Applications, together with the names of three referees, should be received not later than 5 March, 1987, by The Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/353/N. (2106)A

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BIOCHEMIST/Coagulation: Requires MS or Ph.D. in Biochemistry with special emphasis on protein chemistry and enzyme kinetics. A strong background in coagulation biochemistry as well as hands-on experience with diverse instrumentation necessary.

CLINICAL CHEMISTS/Immunochemistry: Several openings requiring BS, MS or Ph.D. in Biochemistry, Immunology, Microbiology, Virology (or related fields). Work on development of Immunoassay diagnostic test kits. Experience with latex a plus.

BIOMEDICAL/CHEMICAL ENGINEER: MS or Ph.D. in Chemical or Biomedical Engineering desired to develop fluid flow synthetic membrane filtration system for use in solid phase immunoassay. Experience in enzyme/protein immobilization and immunology desirable.

Our laboratories are based in ORANGEBURG, NEW YORK, located on the west side of the Hudson River...within 30 minutes of New York City. Interested candidates should forward their resume which must include salary history and requirements, INDICATING AREA OF EXPERTISE, in strictest professional confidence, to: **Personnel Manager, Fisher Diagnostics, 526 Route 303, Orangeburg, New York 10962.** An equal opportunity employer m/f/h. (NW497)A

Fisher Scientific Group, Inc.
Fisher Medical Division

Position for

Research Assistant

with expertise in **immunology/molecular biology.**

Salary in the range of **£12.000—£21.000**

for Paris Laboratory

The successful candidate should be a non-smoking, ties-free, ambitious, young independent scientist with a strong, friendly personality, **wishing to work hard on provocative ideas, capable of supervising and coordinating several research projects.** He should like travel and work abroad. Conversational French an asset.

Send manuscript letter, C.V., photograph and names of 3 referees to *Box W3823 c/o Nature, 4 Little Essex Street, London WC2R 3LF.*

(W3823)A

The CORIELL INSTITUTE FOR MEDICAL RESEARCH (CIMR) in Camden, New Jersey is expanding into a new facility in 1988 on a common campus with the Cooper Medical Center, a 550 bed, full-service hospital and the University of Medicine and Dentistry of New Jersey (UMDNJ). Faculty appointments at UMDNJ are available.

The Institute, while remaining an independent organization, will utilize its new facility, a 43,000 square foot laboratory building that will complement a one year old 42,735 square foot UMDNJ laboratory building and a 24,265 square foot UMDNJ administrative center.

Due to this expansion, CIMR, which conducts studies in

HUMAN GENETICS, CARCINOGENESIS, AGING, ENVIRONMENTAL MUTAGENESIS, DIFFERENTIATION, INFECTIOUS DISEASES
and houses the
NIGMS HUMAN GENETIC MUTANT and **NIA AGING CELL REPOSITORIES**

is **recruiting** all levels of scientists, from **post doctoral fellows through senior faculty** with active research programs in such fields as

MOLECULAR HUMAN GENETICS, CANCER, NEUROBIOLOGY, DIFFERENTIATION

Investigators with records of excellence in other areas are also
invited to apply

Funds for projects and laboratory relocation available
Limited endowment funds available

Qualified candidates may submit their curriculum vitae, a description of proposed research and the names of 3 references to Gerard J. McGarrity, President, CIMR, Copewood Street, Camden, NJ 08103.

CORIELL INSTITUTE FOR MEDICAL RESEARCH

Equal Opportunity in Employment is the policy of the Coriell Institute.

(NW498)A

Senior Fermentation Scientist

£18,000 – £20,000

Beckenham, Kent

Wellcome Biotech, at the forefront in the application of biotechnology to the production of new medical products, has created a new post, Section Head, Fermentation Development. For this, it seeks to recruit a scientist of the highest calibre to lead a highly trained team of 9 studying various problems with microbial and mammalian cell cultures, including:

- the assessment of genetically-engineered cultures to optimise expression systems and also conditions for large scale fermentation. For this work, a number of modern experimental fermenters are available.
- the manufacture of clinical trial materials in 100 and 200 litre fermenters under conditions of Good Manufacturing Practice.

Located in a pleasant parkland estate, you will be working in a just-completed multi-million pound facility equipped with a sophisticated computerised fermenter control system linked to gas analysis by mass spectroscopy.

Candidates should possess a PhD in Biochemical Engineering, Biochemistry or Microbiology together with a successful research and management record in fermentation science.

We offer an attractive salary, dependent on experience together with excellent benefits including five weeks' holiday, pension and life assurance scheme, first class sports and social club facilities and assistance with relocation expenses where appropriate.

Please write with full career details to Mr. Ron Sutton, Senior Personnel Officer, Wellcome Biotech, Langley Court, Beckenham, Kent BR3 3BS, quoting ref. no. 029.

(2161)A

- **A world leader in Biotechnology**
- **Novel therapeutic proteins and vaccines**
- **Established in 1982**
- **A subsidiary of The Wellcome Foundation Ltd**
- **An equal opportunity employer**

WELLCOME **BIOTECH**

CHIEF OF DIVISION OF ANATOMICAL PATHOLOGY — PEDIATRIC PATHOLOGIST.

Immediately opening. Children's Memorial Hospital, a tertiary care major teaching hospital affiliated to Northwestern University is seeking a chief in the Division of Anatomical Pathology. The candidate is required to be board certified in Anatomical Pathology and to have demonstrated excellent ability in surgical pathology, pediatric pathology, and teaching. Must be qualified for Northwestern University faculty position at associate professor or professor level. Salary is negotiable and competitive.

Apply with curriculum vitae, bibliography and names of three references to: **F. Gonzalez-Crussi, M.D., Chairman of Search Committee, Children's Memorial Hospital, 2300 Children's Plaza, Chicago, IL 60614.**
An Equal Opportunity Employer. (NW521)A

CHIEF OF DIVISION OF CLINICAL PATHOLOGY

Immediately opening. Children's Memorial Hospital, a tertiary care major teaching hospital affiliated to North-western University is seeking a chief in the Division of Clinical Pathology. The candidate is required to be Board Certified in anatomical pathology/clinical pathology or clinical pathology. Administrative, clinical, teaching and research responsibilities, and qualified for North-western University faculty position at associate professor or professor level. Salary is negotiable and competitive.

Send curriculum vitae, bibliography, and names of three references to: F. Gonzalez-Crussi, M.D., Chairman, Search Committee, Children's Memorial Hospital, 2300 Children's Plaza, Chicago, IL 60614.

An Equal Opportunity Employer. (NW522)A

POSITION AVAILABLE

RESEARCH IMMUNOLOGIST

sought by Transplantation Unit Laboratories. Applicant should have Ph.D., M.D., or equivalent and considerable experience in experimental immunology as related to transplantation. Appointment as Assistant Professor at Harvard Medical School probably appropriate.

Successful applicant will conduct indepedent research program and some teaching along with collaborative work as desired. Salary appropriate. Women and minorities encouraged to apply. Applications to Henry J. Winn, Ph.D., Transplantation Unit, Massachusetts General Hospital, Boston, MA 02114 before March 1, 1987.

(NW505)A

MOLECULAR MODELLING

A five-year position for a staff scientist is open immediately with experience in the general area of protein structure. Familiarity with molecular dynamics, energy minimization, docking procedures and picture systems would be advantageous. The necessary hardware is available. Information can be obtained from the address below.

The candidate is expected to collaborate with experimentalists in protein engineering but would have ample opportunity to pursue independant research if desired. Salary will be commensurate with qualifications.

Interested applicants are kindly asked to send c.v. and addresses of referees to: Professor Ernst-L. Winnacker, Genzentrum der Universität, c/o Max-Planck-Institut für Biochemie, Am Klopferspitz, D-8033 Martinsried bei München, FRG. Tel: 089/85783941. (W3826)A

Nestlé

For our research department we seek a

NUTRITIONIST/BIOCHEMIST

who will be assigned the following tasks:

- Study of the dietary factors influencing the absorption and utilization of trace elements.
- Development of techniques using stable and radioactive isotopes for the study of the absorption and utilization of iron, zinc, copper and selenium in man and experimental animals.

Qualifications required:
— PhD with experience in trace elements nutrition.

Please send detailed curriculum vitae to:

Mrs. M. Germanier-Audrin, Personnel Services, Research Centre, Nestec Ltd., Ch-1800 Vevey, Switzerland. (W3821)A

Ocean Drilling Program/ Texas A&M University

The Ocean Drilling Program invites applications for the position of Staff Scientist in the Department of Science Operations. Applicants must have a Ph.D. in geology or related field and a strong background in marine geology, preferably in sedimentology. Applicants must also have a demonstrated fluency in written and spoken English. Experience as a seagoing scientist is preferred. Successful applicants will be expected to sail as staff scientific representative on an ODP cruise approximately every 1.5 - 2 years, and coordinate post-cruise scientific research and publication of cruise results. Individual research and interaction with colleagues in the Departments of Oceanography, Geology, and Geophysics are encouraged.

This position is available immediately, and will be filled as soon as possible. Salary will be commensurate with qualifications and experience of applicant. Applicants should forward a curriculum vitae, bibliography, statement of research interests, and names and addresses of four references to: **Employment Manager, Personnel Department, Texas A&M University, YMCA Building, College Station, TX 77843.** Closing date for applications is March 31, 1987.

Equal Opportunity Employer

(NW504)A

PSYCHIATRIC GENETICS PROGRAM

Department of Phsychiatry, Medical College of Virginia/ Virginia Commonwealth University

is recruiting a fulltime

RESEARCH SCIENTIST

interested in applying molecular genetic techniques to neuropsychiatric diseases. This person will join an active research team with a strong emphasis on quantitative genetic methods, with several major psychiatric genetic field studies currently underway. Responsibilities will include supervision of linkage analyses using RFLP's and establishment of immortalized cell lines. The candidate will be strongly encouraged to establish his/her own research program in an area relevant to the molecular biology or neuropsychiatric diorders. Must have Ph.D. or equivalent and advanced research training in molecular genetics. This will be a tenure track position. Send CV and references to **Dr. K. Kendler, Depts. of Psychiatry and Human Genetics, MCV, Box 710, Richmond, VA 23298.** *MCV/VCU is an EEO/AA Employer.* Women and minorities are encouraged to apply.

(NW502)A

ZOOLOGICAL SOCIETY OF LONDON
INSTITUTE OF ZOOLOGY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistant to work on a project examining the role of pheromones in suppressing fertility and growth in primates and rodents. An interest in reproductive biology and experience of behavioural recording techniques desirable. Experience of RIA techniques essential.

The appointment, funded by the Wellcome Trust, is for two years from 1 April 1987 at a salary, aligned to University Lecturer scale, up to £9,495 pa (under review) plus £1,395 London Weighting. Applications with a detailed CV and the names of two referees should be sent to the Establishment Officer, The Zoological Society of London, Regent's Park, London NW1 4RY, by 13 March 1987.

(2143)A

MACQUARIE UNIVERSITY

Sydney — Australia

School of Biological Sciences

MACQUARIE UNIVERSITY RESEARCH FELLOW IN PLANT BIOLOGY

A plant biologist with skills in protein separation and/or molecular biology is required to join a research group studying the "Physiological basis of plant response and adaptation to environmental stress". The successful applicant will take part in a study of the physiology of the heat shock response in plants. Expertise in stress physiology or tissue culture would be an advantage.

Macquarie University Research Fellowships are awarded for two years in the first instance renewable for a final third year after a review of progress by the Macquarie University Research Committee.

Salary range (A$24,013 - A$30,358.

Travel expenses may be provided to the extent of an economy class airfare to Sydney by the most direct route.

For further information contact Associate Professor Barlow on (02) 805 8518 or (02) 868 2889.

Applications, including the names, addresses and telephone numbers of two referees should be lodged with the Academic Staff Office, Macquarie University, 2109 by 11 April 1987.

Equality of Employment Opportunity is University Policy.

(W3820)A

UNIVERSITY OF EDINBURGH
LEUKAEMIA/LYMPHOMA RESEARCH
SENIOR SCIENTIST

Haematology has a vacancy for senior scientist to participate in major collaborative research programme with the MRC Cytogenetics Unit, on human leukaemi and lymphoma, funded initiall for five years by the Kay Kenda Leukaemia Fund. The post advertised is in the area of short- an long-term culture of human marrow and peripheral blood. Existing laboratory facilities are bein expanded and staffing increased The appointment will be to th senior position in the laboratory Applicants should have a PhD an post-doctoral experience in a relevant field. Salary will be in th non-clinical research rang £11,790 — £15,700 (under review).

Further particulars availabl from Dr AC Parker, Haematolog Department, Royal Infirmar Edinburgh. Applications, inclu ing curriculum vitae and names two referees should be sent to D AC Parker, not later than 4t April 1987.

Please quote reference N 5441. (2118)A

MEDICAL RESEARCH COUNCIL
MRC CLINICAL AND POPULATION CYTOGENETICS UNIT
NON CLINICAL SCIENTIST II

Applications are invited for a sho term non-clinical scientific p in the molecular developmen biology group at the above Uni The position, funded in the firs instance for one year is to joi projects already in progress whic aim to elucidate the role of th homeo-box genes in regulatin mouse embryonic developmen Candidates of immediately post doctoral or equivalent status wi be preferred. Experience in on or all of the following technique will be an advantage: cell cultur recombinant DNA, immunolog and protein chemistry. Howeve interested candidates lacking suc experience will be considered, an in-service training will be provide within this expanding group.

Renumeration will be at appropriate point on the scale for university non-clinic academic staff. Further detail from, and applications with name and addresses of two referees, t the Administrator, MRC Clinic and Population Cytogenetic Unit, Western General Hospita Crewe Road, Edinburgh EH 2XU, by 6 March 1987, quotin reference IJ/2/87.

An Equal Opportunit Employer. (2120)A

WASHINGTON UNIVERSITY
MEDICAL SCHOOL

ASSISTANT PROFESSOR

DEPARTMENT OF PHARMACOLOGY

pplications are invited from utstanding candidates for a ighly competitive tenure ack faculty position. Extensive postdoctoral experience mphasizing mechanistic pproaches to pharmacoogical problems are prefered. Send curriculum vitae, eprints, a description of curent/future research plans, nd 3 letters of reference to: **r. Philip Needleman, Deartment of Pharmacology, Vashington University Medical School, 660 S. uclid, St. Louis, MO 63110. AA/EOE)** (NW500)A

OSTDOCTORAL POSIIONS IN NUTRITIONAL CIENCES at the University f Illinois at Urbana-Chamaign. Positions involve funamental and applied nutriion of animals or humans. ndividuals with an M.D. or h.D. in nutrition or allied ields are encouraged to aply. Applications must be reeived by 30 April. Contact: **r. J. A. Milner, Director, Diision of Nutritional Scinces, 451 Bevier Hall, 905 outh Goodwin, Urbana, IL 1801.** *Equal Opportunity/ ffirmative Action Employr.* (NW503)A

Enzymologists/ Protein Chemists

Smith Kline & French is committed to the research and development of ethical pharmaceuticals for human use. Equipped with the latest laboratory facilities housed at our country estate in Hertfordshire we currently seek outstanding Enzymologists and Protein Chemists to join our Medicinal Chemistry Group.

PhD Enzymologists/Biochemists

We are looking for newly qualified PhDs and post-doctoral candidates with experience in enzyme kinetics. The ideal candidate will have had experience in protein isolation and characterisation and be familiar with modern mechanistic enzymology techniques such as PAGE, protein HPLC, FPLC, biological NMR and photoaffinity labelling.

PhD Protein Chemists

We seek newly qualified PhDs and post-doctoral candidates in the field of protein chemistry with experience in manual/automated Edman sequencing, photoaffinity labelling, protein modification and characterisation.

We pay excellent salaries and offer a good range of benefits.

Please send a curriculum vitae quoting reference N/184/PJB to Penelope Bucknell, Personnel Manager, Smith Kline & French Research Limited, The Frythe, Welwyn, Herts AL6 9AR.

SK&F

(2153)A

DIRECTOR

Center for Agricultural Meteorology & Climatology (CAMaC)

Seek applicants to fill major academic and research position in Institute of Agriculture and Natural Resources (IANR), University of Nebraska-Lincoln. Established in 1979, CAMaC currently has a faculty of seven plus several courtesy appointees. Programs include activities in photosynthesis; evapo-transpiration; water use efficiency; surface transfer processes; agricultural micrometeorology; remote sensing; climate impacts assessment; collection; processing and dissemination of weather and climate data and information; integrated pest management and environmental monitoring.

The director will be expected to devote approximately one-half time to administration and programmatic leadership. Considerable time will be devoted to program officers in federal granting and contracting agencies and scholars in other research institutions. The director reports to the IANR Deans and Directors and through them to the Vice Chancellor of the Institute of Agriculture and Natural Resources.

Position requires Ph.D. with experience in application of meteorology/climatology to agriculture and/or natural resources. Must have leadership and administrative potential. Salay and academic rank will be based on qualifications and experience. Apply by April 15 (or until suitable candidates apply thereafter) to:

Dr. Sashi B. Verma, Search Chair
Center for Agricultural Meteorology & Climatology
242 L.W. Chase Hall, University of Nebraska-Lincoln
Lincoln, Nebraska 68583-0728
Phone: (402) 472-6702

Affirmative Action/Equal Opportunity Employer. (NW525)A

ROYAL
BOTANIC
GARDENS
KEW

Keeper of the Jodrell Laboratory

The Jodrell Research Laboratory has an international reputation for the study of plant anatomy, biochemistry, cytology and physiology. The vacancy arises because of the retirement later this year of Professor K Jones.

The Keeper will be responsible to the Director for the overall direction of research and the maintenance of services in the laboratory.

In addition to an established international reputation in an appropriate discipline, candidates should have a broad overall understanding of botanical sciences and the ability to lead and manage a research team.

Salary £18,860-£25,140 (includes £840 Intermediate London Weighting) with starting salary according to qualifications and experience.

RELOCATION EXPENSES UP TO £5000 MAY BE AVAILABLE.

For further details and an application form (to be returned by 3 April 1987), write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours). Telex CSCOMM G 859399.
Please quote ref: S/7148.

An equal opportunity employer

Glaxo Group Research Ltd.

Glaxo

UNIVERSITY OF KENT
AT CANTERBURY ▪ ▪ ▪ ▪

Biochemistry of Candida Albicans

We are seeking to employ a GRADUATE RESEARCH ASSISTANT to undertake a study of the mechanism of protein synthesis in the fungal pathogen *Candida albicans*. Applications are invited from recent graduates with a BSc and/or MSc in biochemistry or related subject. The successful applicant will join a well established group at the University of Kent currently studying various aspects of protein synthesis in the yeast *Saccharomyces cerevisiæ*. The programme is directed by Dr M F Tuite.

This position is funded by Glaxo Group Research with a start date that is negotiable. Appointment will be made on the first three points of the RA1B scale (£7,055 to £8,505), initially for two years, but with the possibility of renewal for a further one year.

Applications including a detailed *Curriculum Vitæ* and the names and addresses of three referees should be sent to Dr M F Tuite, Biological Laboratory, University of Kent, Canterbury, Kent, CT2 7NJ. Enquiries about the post can be made to Dr Tuite at the above address or by telephone on (0227) 66822 ext 3699.

Closing date: 13 March 1987. (2144)A

Positions for
UNIVERSITY GRADUATES
with Bachelors Degree in the
BIOLOGICAL SCIENCES

to do research in the areas of cellular and molecular biology at the — W. Alton Jones Cell Science Center for a period of three years. **Salary ranges from: $8,000.00 — $10,000.00 per year, depending on experience.**

Please send a curriculum vitae to: Personnel Manager,
W. ALTON JONES
CELL SCIENCE CENTER,
10 Old Barn Road, Lake Placid,
New York 12946.

Refer to Job Order 8706. AA/EEO
(NW513)A

POSTDOCTORAL POSITIONS

available to study cellular mechanisms involved in insulin secretion. Biochemical approaches are employed to study the role of lipid-derived mediators in signal transduction events including inositol phosphates, arachidonic acid, and the regulation and function of intracellular Ca^{2+} in insulin secretion.

Please send curriuclum vitae and three references to: **Dr. Michael L. McDaniel, Department of Pathology, Washington University, Box 8118, St. Louis, MO 63110; Telephone: 314–362–7435.** ***Washington University is an Equal Opportunity/Affirmative Action Employer.***

(NW506)A

UNIVERSITY OF OXFORD
DEPARTMENT OF HUMAN ANATOMY

Post of Research Assistant Grade 1A available immediately to work on the mechanism of action of ACTH secretagogues and glucocorticoid hormones in adenohypophysial cells. Funding by the Wellcome Trust for 2 yrs. Expertise with cell culture desirable but not essential.

Send CV and letters of reference to F A Antoni, Dept Human Anatomy, South Parks Rd, Oxford OX1 3QX. Phone: 0865–272178 (2159)A

IMMUNOLOGIST

We are looking for a qualified person to work in an immunoassay laboratory devoted to the quantification of apolipoproteins with an emphasis on automated ELISA. Past experience in immunoassay techniques required. Salary level according to qualifications.

For inquiry contact: **Dr. A.M. Scanu, Director, Lipoprotein Study Unit, University of Chicago, 5841 South Maryland, Box 231, Chicago, IL 60637; telephone 312–962–1775.**

Equal Opportunity Employer
(NW518)A

THE UNIVERSITY OF LEEDS
Astbury Department of Biophysics
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the above post available from 1 April 1987 for a fixed period of up to three years for work on the three-dimensional structure determination of the methionine repressor protein from E. coli involving the use of X-ray crystallography.

A good honours degree and a PhD in a relevant subject area are required. Applicants should preferably have experience in protein crystallography, but small-molecule crystallographers wishing to learn macromolecular techniques will also be considered.

Salary £8,020 - £10,375 (under review) on the R/A IA Range according to age, qualifications and experience.

Informal enquiries may be made to Dr S E V Phillips (tel: (0532) 431751 ext 7581).

Application forms and further particulars may be obtained from the Registrar, the University, Leeds LS2 9JT, quoting Ref No: 42/13. Closing date for applications is 12 March 1987. (2102)A

UNIVERSITY OF BIRMINGHAM
Department of Plant Biology
RESEARCH ASSOCIATE IN PROGRAMMED LEARNING

Applications are invited from graduates to assist in the development of a programmed-learning module in plant genetic resources, specifically in seed conservation. The post is sponsored by the International Board for Plant Genetic Resources and may be held up for up to one year, full-time, or for a longer period on a part-time basis. Ideally the person we require will have a training in biological sciences but with strong interests in innovative educational techniques and/or some experience working with publishers.

Salary will be on the RA IB scale (£7,055 to £9,495) plus superannuation.

For further particulars tel: 021 472 1301 ext 2559, quoting Ref No: K4527. No formal application. Three copies of application including full curriculum vitae and naming three referees to: Assistant Registrar, Faculty of Science, PO Box 363, Birmingham, B15 2TT by 6 March 1987.

An Equal Opportunities Employer. (2105)A

KING'S COLLEGE LONDO
(KQC)
(University of London)
Department of Biology
PLANT BIOCHEMISTRY/ PHYSIOLOGY
POST DOCTORAL RESEARCH ASSISTANT
and
RESEARCH TECHNICIAN

Appointments will be made
work on a project supported
Shell Research Ltd, which is co
cerned with aspects of mobili
ation of storage reserves in germ
nated wheat. The work will i
volve studies of the synthesis a
activity of the enzymes responsib
for mobilisation and will inclu
biochemical and enzymologic
studies combined with molecul
biology (eg manufacture and u
of cDNA probes) and investig
tions of hormonal content. A goo
background in plant biochemist
is required and an interest in pla
physiology, particularly seed ph
siology and biochemistry would
an advantage. The appointmen
would commence in March 19
and would last for 3 years. Startu
salary (post doctoral) in the ran
(£8,020 — £10,375, plus £1,2
London Allowance). Starti
salary (technician) in the Grade
range £5,968 — £6,644 accordi
to relevant qualifications and e
perience, plus £1,365 Lond
Allowance.

Information may be obtaine
from Professor M Black or Dr
Chapman at the Department
Biology, King's College Londo
Camden Hill Road, London W
Tel: 01-937-5411, to whom appl
cations should be sent as soon
possible. There are no applicatio
forms but applicants should send
curriculum vitae and the nam
and addresses of two referees.
(2128)A

IMPERIAL COLLEGE OF SCIENCE & TECHNOLOG

University of London

Lectureship in Organic Chemistry

Applicants will be expected to have doctorate and some post doctoral experience.

The appointment is likely to be made towards the lower end of the scale £9,413 — £17,093 inclusive (currently under review). She-he should be available to start on 1 October 1987.

Applications (including curriculum vitae, list of publications and names of two referees) and requests for further details to:

Professor C W Rees, FRS,
Department of Chemistry,
Imperial College,
London SW72AY.
Telephone 01 589 5111
Ext 4613.
Closing date 10 April 1987. (2156)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Laboratory of Developmental Biochemistry

Applications are invited from postdoctoral candidates for a three year appointment to work on problems concerning the regulation of structure and function of genes during development and on the role of hormones and growth controlling agents. The project will deal with genes coding for egg coat and yolk proteins, the role of steroid hormone receptors and tissue-specific transcription factors in their regulation. There will be ample opportunities for collaboration with other groups interested in similar areas of molecular and cell biology. The work will involve extensive exploitation of the latest techniques of recombinant DNA gene transfection and tissue culture, so that experience in these techniques will be an important consideration.

The salary will be in the range £9,495–£11,790 per annum plus £1,297 London allowance, with superannuation provision (pay award pending).

Applications, including a full C.V. and the names of two referees should be sent to Mr C R Russell not later than 20th March 1987 — quoting reference DBM/872.

Division of Neurophysiology and Neuropharmacology

Applications are invited for a scientific position to conduct research in the general area of ionic conductance in excitable tissue studied at the whole cell and single channel level by use of voltage and patch clamp techniques. Candidates will be expected to pursue their own research programme, and ample opportunity will be available for collaboration within the Institute.

The position might be filled at any level by a career or short term appointment, according to the age and experience of the candidate. The Division is part of the Physiological and Neural Mechanisms Group at NIMR.

The salary will be at an appropriate point on the Council's range equivalent to University non-clinical academic staff scales, plus £1,297 London allowance and superannuation provision.

Applications including a C.V., list of publications, a brief statement of research plans (about 1,000 words), and the names of two professional referees, should be sent to the Director not later than 20th March 1987 — quoting reference NN/872. Informal enquiries to the Director, Dr D A Rees FRS or Dr D R Trentham FRS (Chairman of the Search Committee) are welcome.

The National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA.

The Council is an Equal Opportunities Employer

(2134)A

IMPERIAL CANCER RESEARCH FUND

Molecular Geneticist

required by the ICRF Tumour Immunology Unit based at University College London to join the Hon. Director, Professor N.A. Mitchison. The Unit is concerned primarily with T lymphocyte function, and the scientist would be expected, with appropriate support, to develop own programme of research.

The appointment on a 6 year tenure-track Research Scientist level has a salary range of £14,175 to £17,700. Please quote ref: 57/87

Applications with full CV and names and addresses of three referees should be sent to the **Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX.** Closing date for applications 13th March 1987. (2091)A

RESEARCH SCIENTIST — Inverse Scattering:

Develop theory & application of signal processing, imaging & inversion techniques used in extracting information about material properties of medium from scattering experiments. Perform research in theoretical & applied issues related to multi-dimensional inverse scattering of acoustic & elastic waves. Research on estimation & detection theory, inverse scattering theory, wave propagation in heterogeneous media & multicomponent array processing techniques. Develop, implement & test new algorithms based on various inversion methods & investigate ways of implementing them using advanced computer techniques. 6+ yrs education including PhD or completion of all req's for PhD in electrical engineering, system science or math + 2 yrs research in wave propagation & inverse scattering. Strong theoretical background in digital signal processing & estimation theory. Exp in implementing signal analysis methods on digital computers. 40hrs/wk, $48,000/yr.

Send résumés to: Job Service Technical Unit, Connecticut Dept of Labor, 200 Folly Brook Blvd, Wethersfield, CT06109.

Refer to Job Order #0559856. (NW526)A

MUTATION RESEARCH AT YORK UNIVERSITY IN TORONTO

Two positions are available for **Research Associates** to study the molecular mechanisms of mutagenesis. Projects center on the molecular nature of mutation with the goal of better understanding the origins of spontaneous and induced mutation and their relevance to such areas as cancer and of evolution. The experimental approach involves the cloning and sequencing of mutant genes from bacterial and mammalian cells. Mammalian gene expression and recombination are also being studied. Individuals with strong backgrounds in molecular genetics and/or biotechnology are invited to apply. Salary will commensurate with experience. Letters of application including *curriculum vitae* and the names of three references should be sent to **Dr. B. W. Glickman, Biology Department, York University, North York, Ontario, Canada, M3J 1P3.** *In accordance with Canadian immigration requirements this advertisement is directed to Candian citizens and landed immigrants.*

In addition, applications are also invited from more recent graduates for two **Post Doctoral** appointments. (NW508)A

PALEO-OCEAN/CLIMATE MODELLER:

A new tenure-track faculty position at the Assistant or Associate Professor level is offered with a twelve-month appointment (approximately 1/3 state-funded) in the College of Oceanography at Oregon State University. We seek an ocean/climate modeller interested in the development and application of ocean and/or climate models to understand processes of global paleoclimatic change. Areas of research may include modelling the earth's oceanic, atmospheric, and cryospheric responses to changing boundary conditions, biogeochemical cycles, and the recording of climate signals in the preserved geologic record. The successful candidate is expected to interact with an active research group studying late Neogene to Holocene paleoceanography and paleoclimatology. A Ph.D. in the physical sciences is required. Salary commensurate with experience.

Résumé, names, addresses and telephone numbers of three references must be received by 15 May 1987 by: Dr. Douglas R. Caldwell, Dean, College of Oceanography, CODE-005, Oregon State University, Corvallis, OR 97331.

Oregon State University is an AA/EO employer. (NW517)A

The European Molecular Biology Laboratory, an international research organisation situated in Heidelberg, West Germany, invites applicants for the following position

ELECTRON MICROSCOPY TECHNICIAN

The successful applicant will join our Structure Programme. In addition to routine electron microscopy of biological specimens, using standard techniques, he or she will participate in projects to investigate two-dimensional crystalline layers of membrane proteins and to use specific labelling to give structural information for macromolecular complexes.

Applicants should preferably have experience in electron microscopy with a background in biochemistry, biophysics or biology. Knowledge of English as a working language is required.

An above-average salary will be offered. Certain allowances are payable in addition, depending on personal circumstances. An initial contract of 3 years' duration, which can be renewed, will be offered.

Please write briefly for an application form, quoting reference nº 87/02 to:

EMBL, Personnel
Section, Postfach
10.2209, D-6900
Heidelberg.

(W3832)A

EMBL

CONTESSE

THE UNIVERSITY OF CHICAGO

The Joseph P. Kennedy, Jr. Mental Retardation Research Center and the Department of Pharmacological and Physiological Sciences are recruiting new faculty with research interests related to the molecular and cellular biology of the nervous system.

Applicants should send their curriculum vitae and bibliography, a brief statement of research interests and plans, and the names of three references to:

Dr. Robert L. Perlman, Director
Kennedy Mental Retardation Research Center
Department of Pediatrics
The University of Chicago
5841 S. Maryland Ave., Box413
Chicago, IL 60637

From the time of its original charter, the principle of the University of Chicago has been to appoint scholars without regard to race, color, religion, sex of national origin (NW520)A

POSTDOCTORAL TRAINING IN PLANT MOLECULAR BIOLOGY, MOLECULAR BIOLOGY, BIOCHEMISTRY AND GENETICS

The Plant Gene Expression Center (PGEC) in Albany, California, sponsored by USDA's Agricultural Research Service and the University of California, Berkeley, invites applications from qualified candidates with Ph.D. degrees for ten postdoctoral positions. The PGEC is an interdisciplinary program designed to provide plant agriculture with the technology to maintain and enhance the quality and productivity of the agricultural product.

The PGEC programs include: Auxin-regulated gene expression (A. Theologis), maize transposable elements (B. Baker); monocot transformation (M. Fromm); gene expression in transgenic plants (D. Ow); developmental mutants of maize (S. Hake); flower development (S. McCormick); and phytochrome regulation (P. Quail). Candidates should be familiar with molecular biology, biochemistry, or genetics. Salary is commensurate with experience with a range of $22,000-$27,000. For information on the research program and/or positions, contact: Gerald G. Still (415) 486 3662. To obtain Federal application forms, contact Patricia Roberts (301) 344 3138, USDA, Agricultural Research Service, Room 405, Building 003, BARC-W, Beltsville, Maryland 20705.

An Equal Opportunity Employer

(NW468)A

UNIVERSITY OF GLASGOW

DIRECTOR OF THE COMPUTING SERVICE

The University Court invites applications for the post of Director of the Computing Service. Applicants for the post should be graduates with experience of computer technology and its applications and have experience in the management of computing facilities and the provision of services to academic and scientific users in a University of comparable environment.

Salary will be on Grade IV of the scales for Academic Related Staff (equivalent to professorial range).

Further particulars can be obtained from the Director of Personnel Services, University of Glasgow, Glasgow G12 8QQ, to whom applications (3 copies; 1 copy in the case of overseas applicants), giving the names and addresses of three referees, should be sent on or before 13 March, 1987.

In reply, please quote Ref No: 5886M. (2124)A

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Plant Biology

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a three year NERC-funded postdoctoral position to work on the transition between C metabolism and CAM in the temperate succulent *Sedum telephium*. The work involves the regulation of plant-water relations and gas exchange during the induction and repression of CAM, using infra-red gas analysis, mass spectrometry and pressure chamber techniques.

Salary will be up to £9,000 per annum on the Range IA scale: £8,020 - £12,780 per annum, according to qualifications and experience.

Applications, with curriculum vitae and the names of three referees, should be sent to: Dr H Griffiths, Department of Plant Biology, The University, Newcastle upon Tyne, NE1 7RU not later than 6 March 1987, from whom further information can be obtained. (2115)A

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MEDICAL RESEARCH COUNCIL

Laboratory of Molecular Biology

RESEARCH ASSISTANT

required to join a group working on the chemical synthesis of peptides using modern solid phase techniques. Candidates should have a degree or equivalent qualification in chemistry or a related science. Experience in organic synthesis, especially of peptides, would be an advantage but full training will be given.

Salary will be in the range of £8,020 - £12,280 per annum. Further information on the project is available from Dr R Sheppard at the address below.

Applications including a full cv and the names of two professional referees should be sent by 12 March 1987, quoting reference DIR/2270 to: The Personnel Officer, Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH.

The Medical Research Council is an Equal Opportunity Employer. (2133)A

UNIVERSITY OF ESSEX

Department of Biology

POSTDOCTORAL OR PREDOCTORAL RESEARCH ASSISTANT

Applications are invited for the post of Senior Research Officer/Research Officer for a project, funded by the Cancer Research Campaign, to study the cell and molecular biology of chromosomal proteins involved in the control of cell proliferation and the cell cycle in mammalian cells. Intrasplenic and *in vitro* immunization will be used to prepare monoclonal antibodies to nuclear extracts and partially purified nuclear proteins. Mice with autoimmune diseases will also be used to obtain antibodies that recognise specific nuclear proteins. Appointment will be on Range IA (£8,020 - £12,780 per annum, under review) or Range IB (£7,055 - £9,495 per annum, under review), depending on age, qualifications and experience. The position is for one year in the first instance, renewable for a further two years. Informal enquiries about the post may be made to: Dr Minnie O'Farrell, Department of Biology, tel: (0206) 862286, ext 2022.

Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (R/492/N), University of Essex, Wivenhow Park, Colchester, CO4 3SQ, by 19 March 1987.

(2148)A

UNIVERSITY OF OXFORD

POSTDOCTORAL AND TECHNICAL POSITIONS IN EUKARYOTIC MOLECULAR BIOLOGY

Applications are invited for both a postdoctoral and a technical position funded by a three-year grant from the Wellcome Trust to work with Dr. N. J. Proudfoot. The project related to the mechanism of transcriptional termination of eukaryotic genes transcribed by RNA polymerase II and the role of termination in gene regulation (Johnson et al, Mol. Cell.Biol. 6: 4008, 1986; Whitelaw & Proudfoot, EMBO J. 11: 2915, 1986; Proudfoot, Nature 322: 562, 1986).

It is hoped to fill these positions as soon as possible, preferably soon after Easter 1987. Postdoctoral applicants are preferred with direct experience in recombinant DNA technology and transcription analysis techniques. Technical applicants should have some previous experience in a laboratory situation (preferably a molecular biology laboratory).

Applications and CVs, together with the names of two referees, should be sent to The Administrator, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, as soon as possible. (2137)A

CARIBBEAN REGIONAL DRUG TESTING LABORATORY

MICROBIOLOGIST POSITION

The Caribbean Regional Drug Testing Laboratory in Kingston, Jamaica invites applications to fill the post of Microbiologist.

Duties: Testing and evaluating the microbiological quality of pharmaceutical products, including the development and application of techniques for the assaying of antimicrobials.

Qualifications: MSc or PhD degree in Microbiology. Experience in the analysis quality control of pharmaceutical products preferred.

Applicants should submit curriculum vitae with names and addresses of three references to: The Director, Caribbean Regional Drug Testing Laboratory, c/o Government Chemist's Department, Hope, Kingston, 6, Jamaica, WT.

The closing date for the receipt of applications is 13th March, 1987. (W3833)A

Technician

An opportunity has arisen for a self-motivated technician from the semi-conductor, electron tube or related industry who wishes to gain experience in a new and expanding area of materials development.

Day to day responsibilities will include the operation of semi-conductor processing equipment including sintering, deposition and etching. Clear data records of operating conditions and results will be required. The successful applicant will be expected to take full responsibility for vacuum equipment and chemical work stations. In addition some degree of mechanical tooling and fundamental testing will be required.

Applicants, who should be qualified to at least HND standard, will have experience of at least two of the following:–

Semi-conductor processing
Vacuum technology
Specimen preparation
Materials characterisation
Electronic and computer instrumentation
Precision tooling

Training will be given in any of the above areas which are unfamiliar to the chosen candidate. However, it is expected that applicants will be sufficiently experienced to be able to work with the minimum of supervision.

An attractive salary plus fringe benefits such as free life and medical insurance, non-contributory pension scheme and free lunches are offered in first class working conditions.

Suitably qualified candidates are invited to write to Mrs S. G. Bell enclosing a full Curriculum Vitae, including current salary and outline of present responsibilities.

DE BEERS INDUSTRIAL DIAMOND DIVISION (PTY) LTD.,
Charters, Sunninghill, Ascot, Berks SL5 9PX.

(2109)A

Vacancy Announcement

Associate Vice President for Research

UNIVERSITY OF IDAHO

The University of Idaho invites nominations and applications for the position of Associate Vice President for Research. The Associate Vice President for Research reports to the Vice President for Academic Affairs and Research and is primarily responsible for promoting excellence in research and scholarly activity, enhancing the research environment, providing leadership in broadening and strengthening research and scholarly activity, establishing an administrative structure appropriate for coordinating and supporting research, formulating and administering policy governing research, and representing the university in research-related matters. The Associate Vice President for Research will work closely with the academic deans to coordinate research in the colleges, and with the designated Board of Deans for the various research institutes and centers.

The University of Idaho is a publicly supported comprehensive land-grant institution with principal responsibility for performing research and for granting PhD degrees in Idaho. Regional programs in medical and veterinary medical education in which the state participates are also part of the University of Idaho responsibilities. The UI has programs at 50 sites state-wide. Its land exceeds 8,000 acres. Its nine colleges and Graduate School make the University of Idaho the only comprehensive institution of higher learning in the state.

Applicants or nominees must have an earned doctorate, a distinguished teaching and scholarship record appropriate for appointment as full professor, excellent communication skills, demonstrated ability in obtaining competitive outside funding for research, and administrative experience.

The salary is commensurate with qualifications and experience. The position is available July 1987. Search and selection procedures will be closed when a sufficent number of qualified applicants has been identified, but not earlier than April 10, 1987. Each applicant should submit a letter of application, a current résumé, and the names, addresses, and telephone numbers of three current professional references References will be treated confidentially and contacted only after preliminary screening is completed. All correspondence should be addressed to: **Ms. Patricia Houle, Secretary of the Associate Vice President for Research Search Committee, College of Engineering, University of Idaho, Moscow, Idaho 83843.**

The University of Idaho is an AA/EOE. (NW516)A

Royal Holloway and Bedford New College

UNIVERSITY OF LONDON
EGHAM HILL·EGHAM·SURREY·TW20 0EX

DEPARTMENT OF BIOCHEMISTRY

Postdoctoral Research Assistant

Applications are invited for a postdoctoral Research Assistant to work with Dr W J Owen on a project supported by the Agricultural and Food Research Council concerned with mechanisms of detoxification of xenobiotics in plant cell suspension cultures.

Applicants should have a PhD in Biochemistry, Organic Chemistry or related discipline and preference may be given to those with experience in pertinent chromatographic techniques (TLC, HPLC) and in the use of radio-labelled substances. Previous exprience in the field of pesticide chemistry or biochemistry would also be advantageous.

The salary will be on the RA IA Scale, depending on age and experience, and will include London allowance of £1393 per year.

The position is available immediately for 1 year.

Application forms are available from Mrs D Ward, Personnel Office. Informal enquiries can be made to Dr W J Owen Biochemistry Department on 0784 34455 extension 3563. (2154)A

THE LONDON HOSPITAL MEDICAL COLLEGE

(University of London)

RESEARCH ASSISTANT IN THE DEPARTMENT OF MEDICAL MICROBIOLOGY

Purification and characterisation of a lymphokine

A Research Assistant is required to join a group which is engaged in purifying and characterising a human lymphokine. It is desirable but not essential that the applicant should be experienced in basic protein purification techniques, including HPLC.

The successful applicant will have the opportunity to learn how to use an automated gas-phase protein microsequencer. The post, funded for two years, will be for one year in the first instance, renewable for a further year.

Starting salary: £7,055 plus £1,393 London Allowance.

Curriculum vitae and names and addresses of two referees should be sent to Dr A Coates, Dept of Medical Microbiology, The London Hospital Medical College, Turner Street, London E1 2AD. Telephone 01-377-7643 (direct) or 01-377-7259 (secretary) from whom further details may be obtained. (2130)A

POSITION AVAILABLE

RESEARCH INVESTIGATOR

Perform research on the molecular mechanisms involved in the expression of enzymes that modulate steroid metabolism. Must hold Ph.D. or equivalent degree. Salary is commensurate with qualifications and experience. Please send curriculum vitae and the names and addresses of three persons who can be contacted for reference purposes.

Direct inquiries to:—
Dr. Carl Monder,
The Population Council,
1230 York Avenue,
New York, New York 10021.

(NW515)A

RADIATION BIOLOGIST

to collaborate with NMR spectroscopists and biochemists. Evaluation of the utility of NMR spectroscopy for assessing the state of tumor oxygenation and perfusion and as a basis for the prediction and detection of tumor response to x-irradiation, chemotherapy, and hyperthermia. Position and salary negotiable.

Contact Jerry D. Glickson, Ph.D., Department of Radiology, The Johns Hopkins University School of Medicine, 310 Traylor Building, Baltimore, MD 21205.

(NW514)A

THE UNIVERSITY OF MELBOURNE

CHAIR OF BIOCHEMISTRY

(Re-advertised)

Applications are invited for appointment to a Chair of Biochemistry currently vacant. The University seeks an appointee with an outstanding record in any branch of modern biochemistry.

Salary: $A58,348 per annum (plus a loading of $A5,223 for a medically qualified appointee).

Further information about the position and the Russell Grimwade School of Biochemistry, together with details of application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Registrar, The University of Melbourne, Parkville, Victoria 3052, Australia, or the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 31 May 1987.

The University reserves the right to fill the Chair by invitation.

Equal Opportunity in employment is the policy of the University of Melbourne. (W3829)A

UNIVERSITY OF EDINBURGH

TEMPORARY LECTURER IN ZOOLOGY

(Applied Entomology)

Applications are invited for a temporary Lectureship in Zoology for one year only from 1 October 1987. Placement on the scale of £8,020 to £15,700 will be according to age and qualifications. The main teaching duties are in applied entomology. Further particulars can be obtained from The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS with whom applications by letter (2 copies) including a curriculum vitae and the names of two referees should be lodged not later than 31 March 1987. Please quote reference number 1283. (2059)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified men and women for appointment to the following position:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

VISION RESEARCH GROUP

POSTDOCTORAL FELLOW GRADE I

The appointee will work with Dr Ian Morgan on the characterisation of transmitter systems in the vertebrate retina. Experience with immunohistochemical techniques at the light and electron microscope level, or *in situ* hybridization techniques, is desirable. Familiarity with electrophysiological and biochemical techniques would be an advantage. Enquiries to Dr Ian Morgan, (062) 494671, in the University.

Closing date: 27 March 1987. Ref: BS.29.1.1.

Conditions of Appointment: Salary will be in accordance with qualifications and experience within the range: Postdoctoral Fellow Grade I (at a fixed point) $A24,013 - A$27,507 pa. Appointment will be: Postdoctoral Fellow normally two years, with the possibility of extension to maximum of three years. Grants are provided towards travel and removal. Eligible appointees will be required to join the Superannuation Scheme for Australian Universities. Maternity leave is available. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Applicants should quote the advertisement reference when requesting further particulars and when submitting applicatio[illegible] which should be forwarded t[illegible] the Registrar, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia. Further particulars also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The University is an Equal Opportunity Employer.

(W3830)A

UNIVERSITY OF EDINBURGH

Department of Botany

POSTDOCTORAL RESEARCH FELLOW

Plant Water Relations

Applications are invited for the above post funded by the AFRC to work with Dr J A C Smith on plant responses to water deficits. The work will focus on the role of cell-wall elasticity in osmotic adjustment of leaves to water deficits. Leaf water relations will be investigated at the cellular level using the pressure-probe technique.

The post is tenable for up to three years. Salary will be on the RA1A scale in the range £8,020 to £12,780 per annum (under revie[illegible] according to age and experience.

Applications including a curriculum vitae and the names and addresses of two referees should be sent to Dr J A C Smith, Department of Botany, University of Edinburgh, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH, from whom further details can be obtained (tel: 031 667 1081 ext 3306). Closing date: 16 March 1987. Please quote Ref No: 5442. (2113)A

Tropical Development and Research Institute, London

Fruit and Vegetable Technologist

The TDRI is one of the scientific units of the Overseas Development Administration and collaborates with the developing countries to solve agricultural and health problems. Currently based in London, all TDRI units are moving to Chatham, Kent during 1988.

The Fruit, Vegetable and Root Crops Section is concerned with multi-disciplinary approaches to reducing post-harvest losses and improving the quality of fresh and processed produce, involving improvements in handling, grading storage, ripening, processing, packaging, marketing and quality evaluation. You will develop and manage the R&D programmes and will be required to undertake overseas assignments concerned with the R&D programmes and with the advisory and training activities.

You should have a good honours degree and preferably a PhD in agriculture botany, plant physiology, horticulture or a related subject, together with a proven record of R&D achievement. Overseas experience advantageous.

Salary £15,780-£20,930 (includes £1465 London Weighting), with starting salary according to qualifications and experience.

For further details and an application form (to be returned by 13 March 1987) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 468551 (answering service operates outside office hours), telex 859399 CSCOMM G.

Please quote ref: S(D)677.

The Civil Service is an equal opportunity employer

(2132)A

HYDROLOGIST UC BERKELEY

The Departments of Plant & Soil Biology and Forestry & Resource management are recruiting a Soil and Wildland Hydrologist.

Appointment level may be Assistant, Associate or Full Professor, depending on qualifications and experience. The position is 50% research in the Agricultural Experiment Station and 25% teaching in each of the two departments. Expertise in soil physics and interest in wildland resource management are required. Teaching will include an undergraduate course in wildland watershed management and a graduate course in soil hydrology and/or watershed management. Qualifications include a Ph.D. degree in such fields as soil physics, unsaturated zone hydrology, or watershed management.

Submit letter of application, curriculum vitae, summary of research and teaching objectives and the names of three referees to **Prof. J.G. McColl, Chair, Search Committee, Soil and Wildland Hydrologist, 108 Hilgard Hall, University of California, Berkeley CA 94720 by May 1, 1987. The university of California is an Equal Opportunity/Affirmative Action Employer.** (NW524)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
RESEARCH BIOCHEMIST SURGICAL UNIT

Applications are invited from biochemists who hold a PhD and who wish to pursue a research career within a clinical department. The successful applicant will initially be involved in a multidisciplinary investigation into the value of photochemotherapy in the treatment of cancer. Experience in porphyrin chemistry is desirable but not essential. It is expected that he/she will be involved in and supervise other projects within the department and assist in the teaching of undergraduates and postgraduates.

Salary: £8,020 — £12,780 plus £1,393 London Weighting (Pay award pending).

Informal enquiries may be made to Professor NS Williams, Tel 01 377 7079 (direct line). Applications to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD within 14 days. (2129)A

MRC CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow
Middlesex, HA1 3UJ

POST DOCTORAL NON-CLINICAL SCIENTIST

A post-doctoral research worker is required to join a team studying immunoregulation by antigen-presenting cells. The project will investigate the function of dendritic cells in reactive arthritis in mice and man. Experience in microbiological techniques, tissue culture and animal work will be an advantage.

The appointment is funded by the Arthritis and Rheumatism Council and will be for 3 years in the first instance. *Salary will be within the range £10,888 — £11,763 (inclusive of London Weighting).*

Informal enquiries may be made by contacting Dr. S. Knight 01-864-5311 Ext 2613.

Application forms and further details available from **Jean Cottam, Personnel Section, Extn 2673 Quoting Ref 131/1/ARC.** *Closing date for completed applications 12th March 1987.*

The Medical Research Council is an Equal Opportunity Employer. (2103)A

Royal Holloway and Bedford New College
UNIVERSITY OF LONDON
EGHAM HILL·EGHAM·SURREY·TW20 0EX

DEPARTMENT OF BIOCHEMISTRY

Postdoctoral Research Assistant

Applications are invited for a postdoctoral Research Assistant starting as soon as possible to work with Dr J R Bowyer on herbicide interactions with chloroplast Photosystem II. The project is funded by Shell Research Ltd and will run for an initial period of 2 years.

Applicants should have a PhD in histochemistry with experience in protein chemistry including peptide fingerprinting and analysis, and should preferably have an interest in photosynthetic electron transport.

The salary will be on the RA IA Scale, depending on age and experience, and will include London allowance of £1393 per year.

Application forms are available from Miss K Clarke, Personnal Office. Informal enquiries can be made to Dr J R Bowyer Biochemistry Department on 0784 34455 extension 3803. (2157)A

UNIVERSITY OF STOCKHOLM WENNER-GREN INSTITUTE

Department of Ultrastructure Research

ELECTRON MICROSCOPE

Applications are invited for a 2+2 year postdoctoral position to work within the field of biological ultrastructure. The position includes some teaching and administration in an EM-laboratory, which is well equipped for TEM including X-ray microanalysis and cryotechniques. Experience in at least some of these fields is desirable. Salary within the range of £12,000 - £15,000 per annum.

Applications with a curriculum vitae and a statement of scientific achievements and intentions should be sent to: **The Registrar, Stockholm University, S-106 91 Stockholm, Sweden,** Ref No: 109/87 before March 23. Names and addresses of two referees are recommended. Further information is available from Dr. B. Afzelius, Wenner-Gren Institute, University of Stockholm, 106 91 Stockholm. Tel No: 8 - 16 40 91. (W3825)A

POSTDOCTORAL POSITION IN MOLECULAR NUTRITION

A postdoctoral position is available at the Biotechnology Laboratory of EUROLYSINE, a research joint-venture between AJINOMOTO and ORSAN, on the Orsay campus, 20km south of Paris, to study the digestibility and absorption kinetics of amino-acids and related polypeptides in *in vitro* and *in vivo* model systems.

The group is interested in developing new molecules for animal feed purpose, and close contact is expected with the genetic engineering team located in the same building.

The suitable candidate will possess a PhD in biochemistry with a good knowledge of protein chemistry. The position is for two years initially and offers a good opportunity to train in molecular nutrition.

Applications including CV and two letters of reference should be sent to: Dr B. Jarry, Eurolysine Research Laboratory, Centre Scientifique d'Orsay, Bâtiment 403, 91405 Orsay Cédex, France. (W3822)A

THE UNIVERSITY OF OXFORD

NUFFIELD DEPARTMENT OF ORTHOPAEDIC SURGERY

Applications are invited for a research position to work on the basic physiological defects that result in crippling conditions of soft tissue ossification. The project is sponsored by the National Fund for Research into Crippling Diseases and candidates should have experience, preferably postdoctoral, in biothemical and cell biological research.

Salary will be on the appropriate scale for Research Staff, according to age and experience.

Appointment available initially ten months from March 1987.

Applications including a curriculum vitae and names and address of two referees should be sent a s a p to the Secretary, MRC Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford OX3 7LD. (2150)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF MEDICINE

Applications are invited for the post of Research Assistant/Senior Research Assistant to work with Dr J M Rhodes in the Department of Medicine on a project to study the structure and function of colonic mucus glycoproteins. Applicants should either be in the process of submitting a PhD thesis or be good honours graduates with some relevant laboratory experience.

The post is tenable for one year in the first instance at an initial salary within the range £7,055–£8,020 per annum (under review).

Applications, together with the names of three referees, should be received not later than 16th March 1987, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained.

Quot ref RV/358/N (2151)A

RE-ADVERTISEMENT

THE SCHOOL OF PHARMACY

(University of London)

SENILE DEMENTIA THERAPIES

A two-year postdoctoral research assistant post is available as part of a multidisciplinary project. Candidates must be experienced techniques of animal learning and memory.

Salary on the IA Scale, to start by 1 May 1987. Applications with two copies of curriculum vitae to: Personnel Secretary, The School of Pharmacy, 29/39 Brunswick Square, WC1N 1AX. Previous applicants need not reapply. Further details can be obtained from Dr S File at the School. (2145)A

UNIVERSITY OF EDINBURGH

Department of Biochemistry

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a postdoctoral research Fellow to work with Simon van Heyningen and John Phillips on the effect of tetanus toxin on adrenal cells. This research has been planned with a view to investigating both the secretory mechanism of these cells, and the mechanism of action of the toxin.

The position, which is available for three years, is financed by a grant from the Wellcome Trust. The starting salary will be not more than £9,495 (under review). Further details can be obtained from Dr van Heyningen or Dr Phillips, Department of Biochemistry, Hugh Robson Building, George Square, Edinburgh EH8 9XD, (or tel: 031 667 1011, ext 2385). Please apply by 23 March, 1987 to: Dr S van Heyningen at the above address. Enclose a full curriculum vitae and the names of at least two referees. Please quote Ref No: 5444. (2139)A

THE CARDIOTHORACIC INSTITUTE

(University of London)

Department of Cardiac Medicine

TECHNICIAN

required to support a small group using isolated single heart cells to investigate receptor changes during heart failure. The successful applicant will be trained in the techniques for cell isolation which are well established at the Institute, and will be required to supply the group routinely with freshly prepared cells. There may also be scope for the applicant to pursue some independent research.

The position is funded for three years. Salary on Whitley Council Junior MLSO 'B' scale according to age and experience. Further details may be obtained by telephoning Dr S Harding (01 486 3043).

Applications (2 copies) to include a curriculum vitae and the names and addresses of two referees, should reach the Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP no later than 6 March 1987, quoting CM20. (2117)A

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nature

PLANT·&·ANIMAL BIOTECHNOLOGY

AN INTERNATIONAL CONFERENCE & EXHIBITION

FIRST ANNOUNCEMENT:

Nature's 9th International Conference and Exhibition

KENSINGTON TOWN HALL, LONDON

July 2 and 3

Conference fee £185 plus VAT to include all sessions, coffee, lunch, tea and documentation. For further information call:

Za Dale on (01) 836 6633.

Or write to

Nature, 4 Little Essex Street, London WC2R 3LF, UK.

INSTITUTE OF UROLOGY

(University of London)

POSTDOCTORAL RESEARCH FELLOW

To study the growth and differentiation of urothelium in vitro and its applications to reconstructive surgery and toxicity testing. Three year appointment on University non-clinical academic scale according to experience.

For further information contact: Dr Irene Leigh on 01 377 7749 or Dr John Masters on 01 836 9611 ext 59. Applications to the Administrator, Institute of Urology, 172 Shaftsbury Avenue, London WC2H 8JE by 27 March 1987. (2104)A

INSTITUTE OF ORTHOPAEDICS

ROYAL NATIONAL ORTHOPAEDIC HOSPITAL
Brockley Hill, Stanmore,
Middx HA7 4LP

RESEARCH ASSISTANT IN BIOCHEMISTRY

Candidates should have graduated recently in biochemistry or a related subject, preferably with interests in biomedical research and drug action. The position is available for 3 years, renewable annually. The successful applicant will be encouraged to register for a higher degree.

This is a collaborative project, investigating the structure and function of plasma fibronectin in rheumatoid arthritis and its interacts with anti-rheumatic drugs. Relevant technical experience in protein handling or the assay of drug levels in body fluids would be an advantage.

Starting salary in range £8,448–8,923 (inc London Weighting).

Application forms from Institute Secretary, 01 954 2300, ext 379. (Closing date 3 weeks from publication) (2160)A

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

DEPARTMENT OF CHEMISTRY

A POST-DOCTORAL POSITION is available for September, 1987. Research involves synthesis and study of binuclear and polynuclear complexes (especially copper) as protein models and potential redox catalysts. Experience in electrochemistry, magnetochemistry and kinetics is desirable.

Send CV and names of three referees to:

Dr. L.K. Thompson
Department of Chemistry
Memorial University of Newfoundland
St. John's Newfoundland
Canada, A1B 3X7 (NW509)A

THE UNIVERSITY OF SHEFFIELD

Research Institute for Photosynthesis

1. RESEARCH ASSOCIATE
2. RESEARCH TECHNICIAN

Applications are invited for the above posts tenable for one year in the first instance with the possibility of extension for a further two years. The posts are funded by Shell Research and will be based in the Institute located in The University's Botanical Gardens, under the dirction of Professor David Walker, to research regulation and measurement of photosynthesis in collaboration with the Shell Sittingbourne Research Centre.

1. Candidates should possess or be about to obtain a PhD in Biology or Biochemistry or any relevant discipline. Appointment will be on the scale for Research and Analogous staff Grade 1A points 1-4 £8,020 to £9,495 pa (under review) according to age, qualifications and experience.
2. Appicants should hold an HNC or equivalent in Biological Sciences. Knowledge of biochemical and gas exchange techniques and previous experience will be an advantage. Salary will be on the scale for Technician Grade 5 £7,274 - £8,588 pa.

Further particulars are available from Dr M Sivak or Dr R Leegood Tel: (0742) 768555 ext 6404. Applications (for post 1, 6 copies) including a curriculum vitae and the names of 2 referees should be sent to the Personnel Department, The University of Sheffield, Sheffield S10 2TN to arrive by 31 March 1987. Please quote Ref: S2075/N. An Equal Opportunity Employer. (2138)A

VISITING SCIENTIST POSITIONS

are available for new and established Ph.D.'s and/or M.D.'s in the areas of cell and molecular biology. Some experience in either area is desirable but not essential. Appointments will be made for one to three years; annual salary of $20,000.00 to $25,000.00 dependeing on qualifications and experience.

Please send curriculum vitae and three names of references to: Personnel Manager,
W. ALTON JONES
CELL SCIENCE CENTER,
10 Old Barn Road,
Lake Placid, New York 12946.
PLEASE REFER TO JOB ORDER 8705. AA/EEO (NW512)A

SOFTWARE SPECIALIST FOR THE INFRARED SPACE OBSERVATORY

at Max-Planck-Institut für Kernphysik, Heidelberg, Federal Republic of Germany.

The Infrared Space Observatory (ISO) is an ESA-approved satellite project scheduled for launch in 1992–1993 as a follow-up mission to the Infrared Astronomical Satellite. The Max-Planck-Institut für Kernphysik is participating in ISO through its contribution towards the development of ISOPHOT, a multiband imaging spectro-photometer which will be one of the four focal plane instruments aboard the spacecraft. A vacancy exists for a software specialist to assist in the design and implementation of a computer system for scientific analysis of data from ISOPHOT. The successful applicant will participate in the architectural and detailed design of the scientific analysis software (including software for the real-time processing of data received in the ISO ground station), and will be responsible for the verification and documentation. This work will be done in collaboration with scientific institutes in Denmark, England, Ireland, Spain & West Germany. In addition, he or she will be encouraged to pursue a program of astronomical research in preparation for the ISO mission.

The post is available immediately. Applications are invited from observational astronomers with experience in software development. Candidates should include a curriculum vitae, the names of two references, and a brief description of their present work and relevant experience.

While the language at the MPI für Kernphysik is obviously German, ISOPHOT is an international collaboration requiring a solid working knowledge of English. Although the post is limited to a period of three years, it is envisaged that the successful candidate will eventually take up a post as an ISOPHOT instrument specialist at the ISO ground station (at a still undecided location) for the two year duration of the mission. Renumeration will be within range RAT IIa of the salary scale of the German civil service.

The letter of application should be addressed to: **Max-Planck-Institut für Kernphysik, Postfach 103980, 6900 Heidelberg 1, BRD. More detailed information can be obtained from Prof. Dr. H.J. Völk, Phone: 06221–516295.** (W3831)A

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London WC2R 3LF
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65 Bleecker Street
New York, NY 10012
Tel: (212) 477-9600

TORONTO OFFICE
Peter Drake & Associates
17 Pine Crescent
Toronto, Ontario M4E 1LI
Tel: (416) 690-2423

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MEDICINE

(WGH)

RHEUMATIC DISEASES UNIT

CELLULAR IMMUNOLOGIST/ BIOCHEMIST

Applications are invited from postdoctoral cellular immunologists/ cell biologists/molecular biologists/ biochemists to join a cytokine research group investigating the role of Interleukin 1, tumour necrosis factor and other cytokines in the pathogenesis of rheumatic diseases. Funding is for three years from a five-year ARC Programme Grant, with the salary on the 1A scale (£9,495–12,780 pa) pending 1986 revision.

Further information may be obtained from Professor G Nuki or Dr G W Duff, Rheumatic Diseases Unit, Northern General Hospital, Ferry Road, Edinburgh EH5 2DQ (telephone 031 332 2525).

Applications, including CV and names and addresses of two referees, to Professor Nuki by 12 March 1987. Please quote reference number 5440. (2093)A

ASSISTANTSHIPS

UNIVERSITY OF YORK

Department of Biology

Applications are invited for a research assistantship for a period of three years from 1 September 1987, to work on the isolation and characterisation of genes responsible for resistance to certain anti-cancer drugs in mammalian cell cultures. Candidates should expect to graduate with at least an upper second Honours degree with specialisation in Biochemistry or Molecular Genetics. The successful applicant will be eligible to apply for higher degree registration.

Starting salary £7,055 per annum, with USS. (currently under review).

Three copies of applications, with full curriculum vitae and naming two referees, should be sent by 29 March 1987 to: Registrar's Department (Appointments), University of York, Heslington, York YO1 5DD. Further particulars are available. Please quote advertisement Ref No: 7/6238. (2142)P

THE UNIVERSITY OF LANCASTER

Department of Environmental Science

RESEARCH ASSISTANT IN ATMOSPHERIC CHEMISTRY

Applications are invited for a postgraduate research assistantship for one year, to work with Dr. C. N. Hewitt on the identification and analysis of organic pollutants in ambient air and precipitation. Laboratory experience in gas chromatography is desirable. Informal enquiries to: Dr. Hewitt on 0524 65201 ext 4832/4677. Salary will be on the bottom point of Research IB scale (£7,055 - £9,495 pa under review). Further particulars and application form are available from the Establishment Office (quoting Ref: L323/B) University House, Bailrigg, Lancaster LA1 4YW, where applications (5 copies) should be sent to arrive not later than 11 March 1987. (2119)P

STUDENTSHIPS

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Medicine (Rheumatology)

PhD RESEARCH STUDENTSHIP

Applications are invited for a PhD studentship to work on mechanisms of antigen presentation in the induction of immune responses in mice, funded by Roche Products Limited. The student will join an established research group studying cellular and molecular requirements for T cell activation.

Applicants should possess or expect to obtain at least an upper second class Honours degree in Biochemistry, Biology or a related subject.

Applications, together with the names of two referees, or enquiries for further particulars, should be addressed to: Dr J A Goodacre, Department of Rheumatology, Royal Victoria Infirmary, Newcastle upon Tyne, NE2 4LP. (Tel No: 091 2328511 ext 4181). (2116)F

THE LONDON HOSPITAL MEDICAL COLLEGE

(University of London)

STUDENTSHIP IN DEPARTMENT OF MEDICAL MICROBIOLOGY

Improved DNA sequencing techniques

Applications are invited for a Research Student to join a group which is engaged in improving DNA sequencing technology. The applicant should possess a First or Upper Second class Honours degree in a relevant biological discipline such as Biochemistry or Biotechnology.

The post is funded for three years. The applicant is expected to register for an MPhil in the first instance and then a PhD. The Studentship is currently £3,366 per annum (exempt from tax and National Insurance).

Curriculum vitae and the names of two referees should be sent to Dr A Coates, Dept of Medical Microbiology, The London Hospital Medical College, Turner Street, London E1 2AD. Telephone 01-377-7643 (direct) or 01-377-7259 (secretary) from whom further details may be obtained. (2131)A

ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

ANATOMICAL SOCIETY GRADUATE STUDENTSHIPS

Applications are invited for Studentships tenable in the Anatomical Sciences for a period up to three years. Nominees would be expected to work for a higher degree by research. Candidates must be graduates of British or Irish Universities and must have arranged sponsorship with a Department of Anatomical Sciences in the UK or Ireland. The stipend will be commensurate with basic Research Council rates.

ANATOMICAL SOCIETY SENIOR VISITING FELLOWSHIP

Applications from Overseas Scientists are invited for one Fellowship tenable for a period of less than a year in a Department of Anatomical Sciences in the UK or Ireland. Applicants should be of postdoctoral or comparable status and must have arranged sponsorship in the Department in which they intend to work. Some assistance towards travel and subsistence will be provided.

Further particulars of the Appointments should be obtained from the Honorary Secretary, Professor J A Gosling, Department of Anatomy, Stopford Building, University of Manchester, Oxford Road, Manchester, M13 9PT, to whom applications should be sent by July 15th 1987. Applications for studentships must be accompanied by sealed letters of support from two referees. (2122)F

UNIVERSITY OF LIVERPOOL

Department of Physiology

RESEARCH STUDENTSHIPS

Applications are invited for studentships tenable for three years from students who have or expect to obtain a first or upper second class honours degree. The following projects will be offered:

Professor O H Petersen — Control of potassium ion channels in insulin-secreting cells. Professor G J Dockray — Phosphorylation of gut hormones. Dr R Dimaline — Physiology and biochemistry of VIP-like peptides. Dr R Burgoyne — The role of the cytoskeleton in the control of axonal growth. Mr D V Gallacher — Neurotransmitter regulation of electrolyte and water fluxes across transporting epithelia. Dr R J Cayton and Dr D Taylor — Physiology and pharmacology of hypothalamic neurons receiving input from cutaneous and visceral receptors. Dr H J Tracy — A study in anesthetised ferrets of the neurohormonal mechanisms involved in the release of antral gastrin by distension.

Further details may be obtained from Dr R D Burgoyne (Tel: 051 709 6022 ext 2446). (2125)F

UNIVERSITY OF CAMBRIDGE

AFRC UNIT

Department of Zoology

RESEARCH STUDENTSHIP IN INSECT NEUROPEPTIDES

Applications are invited for on research studentship, tenable for years, commencing October 1987 Candidates will be expected t register for a PhD degree an must have achieved a first-cl or upper second-class deg in Biochemistry, Chemistry, Ph iology or Zoology.

The research project wi examine peptides as neurotransmitters and neuromodulators i the insect nervous system usin a multidisciplinary approac combining biochemical, physic logical and immunocytochemic techniques.

The project will be supervise by Dr P D Evans. Applican should write to: The Secretar AFRC Unit, Department of Zo logy, University of Cambridg Downing Street, Cambridge CB 3EJ, UK as soon as possibl including a full curriculum vit and the names of two referees. (2121)

INSTITUTE OF HORTICULTURAL RESEARCH

Littlehampton, West Sussex

Applications are invited for a 3 year Agricultural and Food Research Council Studentship to study the application of molecular biologist techniques to study changes in gene expression associated with floral induction.

Qualifications: 1st or 2.1 hon ours degree in a biological scienc Candidates should have a interest in the biochemical regula tion of plant development.

Further details and applicatio form from the Personnel Office IHR, Bradbourne House, Ea Malling, Kent. (2141)F

FELLOWSHIPS

ST JOHN'S COLLEGE OXFORD

The President and Fellows of St John's College invite applications for a Senior Visiting Research Fellowship for the academic year 1988-89, or part thereof. The Visiting Fellowship is intended to offer a scholar of outstanding distinction, either from abroad or from the United Kingdom, an opportunity to pursue his or her own study or research as a member of the College. The Fellow will be expected to reside in Oxford during term, and will have the right to lunch and dine in College free of charge. Accommodation free of charge will be available for married or unmarried Fellows.

Applications should be addressed, not later than 31 March 1987, to the College Secretary, St John's College, Oxford, OX1 3JP, with a statement of the research or other work proposed during the visit.

(2108)E

THE RANK PRIZE FUNDS

TWO RESEARCH INITIATIVES IN

Regulatory Control of Nitrogen or Protein Metabolism in Plants, Animals or Man

The Trustees of The Rank Prize Funds wish to establish two three year research initiatives for younger scientists (up to the age of 40 although this is not to be strictly applied) to enable them to further their research in this area. It is anticipated that the successful applicants will already have or have promise of appointments and established bases for research and supporting evidence to this effect should be submitted.

Initial applications should provide a brief outline of the proposed research programme, using as a base an annual sum not greater than £25,000. These Funds will be available for supportive salaries and or equipment. Details of past research work should be included.

Applications will be considered on a competitive basis by the Trustees' Advisory Committee on Nutrition. Short listed candidates will be provided with further details and should expect discussions with Members of the Committee.

Applications should be addressed to Miss Judith Barnes at The Rank Prize Funds, 12 Warwick Square, London, SW1V 2AA to arrive no later than 30th April.

(2135)E

continued on page 16

CELLTECH PhD STUDENTSHIPS

Celltech Limited offer two PhD Studentships for October 1987 to be selected from the following projects:-

1. Chemical modification of monoclonal antibodies to produce novel therapeutic molecules
2. Purification and cloning of novel lymphokines for therapeutic application
3. Protein Engineering of human fibronectin
4. Analysis of *cis* and *trans* factors in transcription and translation in mammalian cells
5. Homologous recombinant and site directed integration in mammalian cell chromosomes
6. Expression of recombinant immunoglobulin genes in mammalian cells
7. Enhancement of the secretion of proteins from eukaryotic cells
8. Identification of genes and processes involved in proliferation of eukaryotic cells

Applications are invited from graduates or prospective graduates with a relevant upper second class or first class honours degree for these positions.

Please send a C.V. including the name of two referees and a statement of research interests to: Dr. T.J.R. Harris, Director of Studies, Celltech Limited, 250 Bath Road, Slough, Berkshire, SL1 4DY.

(2152)F

CELLTECH

UNIVERSITY OF EDINBURGH

Centre for Reproductive Biology, Edinburgh

RESEARCH STUDENTSHIP

Applications are invited for a University funded three year studentship to start in October 1987 for research into the role of inhibin and related peptides on the regulation of ovarian activity in sheep and women. Additional information from: Professor David T Baird (Tel No: 031 229 2575 ext 28). Applicants should hold or expect to obtain a First or Upper Second Class Degree in Biological Sciences.

Applications, including full curriculum vitae and the names of two referees should be submitted to: Department of Obstetrics and and Gynaecology, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW. Closing date: 13 March 1987.

(2146)F

UNIVERSITY OF KEELE

SHELL UK LTD

POSTGRADUATE STUDENTSHIP

Applications are invited for a three-year postgraduate studentship, funded by Shell UK Ltd, leading to a PhD degree. The topic is 'Controls on clastic hydrocarbon reservoir development in the Dinantian and Namurian of Central and Northern England' and will be supervised by Dr B M Besly, to whom initial enquiries should be addressed.

The stipend will be enhanced above that for Research Council Studentships and the successful applicant will be expected to commence the study at a mutually convenient date prior to 1 October, 1987. Closing date for applications is 31 March, 1987.

(2140)F

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

Department of Biochemistry

POSTGRADUATE OPPORTUNITIES

The department invites applications from students who expect to obtain a First Class or Upper Second Class Honours degree this summer, and also from mature students, for postgraduate studentships available in its following research groups: *Membrane Biochemistry, Plant Biochemistry, Terpenoid Biochemistry, Microbial Degradation, Animal Nutrition and Soil Science.*

Details of the research arears are available on request, and we welcome enquiries to the Postgraduate Admissions Tutor, Department of Biochemistry, University College of Wales, Aberystwyth, Dyfed SY23 3DD.

(2136)F

IMMUNOLOGY TRAINING PROGRAM POSTDOCTORAL FELLOWSHIPS

Postdoctoral positions are available within the Department of Microbiology and Immunology at Duke University Medical Center, supported by NIH Institutional Training Grants. Some of the research interests of the 38 faculty members are:

D. Bernard Amos: Immunologic recognition of human and murine histocompatibility antigens of tumors and allografts; HLA polymorphisms

Yair Argon: B cell differentiation and the control of antibody secretion

Andrew Balber: Regulation and function of African trypanosome surface glycoproteins

R. Randal Bollinger: Transplantation immunology, induction of immunological tolerance

Rebecca H. Buckley: Human T and B cell development and aberrations in their development and regulation

Mary Carrington: Regulation of HLA class II gene expression

Ronald B. Corley: Gene expression in differentiating B lymphocytes and B cell subsets; function(s) of class II molecules in B lymphocyte activation

Peter Cresswell: Biosynthesis and genetic regulation of the glycoprotein HLA antigens

Jeffrey R. Dawson: Role of NK cells in regulating lymphocyte differentiation and function

Olja Finn: T-cell repertoire in human anti-allograft and anti-tumor responses

Barton F. Haynes: Human T cell biology, thymic microenvironment-thymocyte interactions and human T lymphotropic retroviruses

Donna Kostyu: Human MHC genes, definition and participation in immune responses

Elwood Linney: Gene expression in mouse teratocarcinoma

Richard Metzgar: Basic and clinical studies of membrane associated antigens of normal and malignant human and simian cells

Frances E. Ward: Human histocompatibility genes, polymorphism and regulation of expression

Send curriculum vitae with a description of research interests and goals, names of references, and indicate faculty member(s) of interest to: **Dr. Frances E. Ward, Postdoctoral Coordinator, Box 3010, Duke University Medical Center, Durham, North Carolina 27710**

AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

DUKE UNIVERSITY MEDICAL CENTER

(NW499)E

NATIONAL CANCER INSTITUTE

National Institutes of Health

CANCER PREVENTION FELLOWSHIP PROGRAM (CPFP)

The Division of Cancer Prevention and Control is accepting fellowship applications for a proposed August 31, 1987, entry into CPFP. Funding permitting, up to ten individuals may be accepted for three years of training. Requirments are: M.D., D.O., or an accredited doctoral degree in a discipline related to cancer prevention and research (medicine, biomedical or behavioral research, nutritional science) or equivalent; academic-professional excellence supported by official transcripts; four letters of reference; and U.S. citizenship or a resident alien eligible for citizenship within four years at the time of application. *Current salary ranges for an M.D./D.O. or a Ph.D., respectively, are $26,000 — $35,000 or $18,000 — $31,000 per annum.* Send a postcard with your name and address to **Ms. Nancy E. Garner, NIH/NCI/DCPC/CCAB, Blair Building, 9000 Rockville Pike, Room 4A01, Bethesda, MD 20892–4200**, or call (301) 427-8788 for application and information packet.

DEADLINE FOR RECEIPT OF APPLICATIONS IS MARCH 16, 1987.

An Equal Opportunity Employer.

(NW507)E

UNIVERSITY OF EDINBURGH

DEPARTMENT OF PRECLINICAL VETERINARY SCIENCES

POSTDOCTORAL RESEARCH FELLOWSHIP IN NEUROSCIENCE

A Postdoctoral Research Fellow is required to join a MRC Programme Grant team studying the anatomical and functional properties of identified neurons in the spinal cord Experience of in vivo neurophysiological techniques is required, preferably including intracellular as well as extracellular microelectrode methods and ionophoresis. The post will be available from 1st May, 1987 and is funded to 31st December, 1988 on the grade 1A Scale (£8,020 — £12,780) with a possibiliy of renewal for a further period of up to 5 years. Further information may be obtained from, and applications (including a cv and the names of two referees) should be sent to: Professor A G Brown, Department of Preclinical Veterinary Sciences, University of Edinburgh, Summerhall, Edinburgh, EH9 1QH.

Plese quote reference No 5439.

(2092)E

YALE UNIVERSITY POSTDOCTORAL FELLOWSHIP

This position is available immediately for the analysis of polyamine dependence in *E. coli.* Detailed genetic and physiological approaches will be used to determine which cellular functions are sensitive to very low polyamine levels.

Applicants should send a c.v. with names of references to: **Dr. Brooks Low, Radiobiology Laboratories, Yale Medical School, 333 Cedar Street, New Haven, CT 06510.**

(NW511)E

BIOTECHNOLOGISTS!

What's going on in the world's fastest-growing scientific discipline?

To find out, send for a sample copy of BIO/TECHNOLOGY, the International Monthly for Industrial Biology, to

Felicity Lyons, Macmillan Journals, 4, Little Essex St.
London, WC2R 3LF

or

Paige Beaver, Bio/Technology, 65, Bleecker St.,
New York, NY10012, USA

CHRIST'S COLLEGE CAMBRIDGE

AMERSHAM SENIOR RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for a Senior Research Fellowship in Biochemistry at Christ's College, founded by Amersham International plc.

The Fellowship is intended for a biochemist of exceptional ability who is well qualified for an academic, industrial, or research career, but who does not hold a tenured post at the time of application; it is also suitable for someone on leave from a tenured post who wishes to concentrate on research for a period. It is hoped that the successful candidate, who would normally not be aged over 40, would take up the Fellowship not later than 1 October 1987 and would hold it for a maximum of four years. Although the choice of a research topic would be left to the candidate, preference will be given to candidates who wish to carry out research involving radioactively labelled compounds, or applications technology. Candidates will normally be expected to work within the University Department of Biochemistry.

The stipend offered is incremental within the range of the University Lecturers' scale (£10,865 — £15,700), the starting point depending on age and experience; for an unmarried Fellow, accommodation in College may be available, with some adjustment in stipend. The Fellow would be entitled to seven free meals per week (either lunch or dinner) and a Commons Allowance.

Forms of application may be obtained from Professor Sir Hans Kornberg, FRS, The Master, Christ's College, Cambridge CB2 3BU, to whom completed applications should be returned by Friday 10 April 1987. (2147)E

The Wellcome Trust

Wellcome Research Fellowships to Scandinavia 1987-1988

Applications are invited from post-doctoral research workers currently working in the United Kingdom and Ireland for the award of Wellcome-Scandinavian Research Fellowships for 1987-1988, to start in October 1987.

The object of these fellowships is to encourage working visits to Denmark, Finland, Norway or Sweden by investigators in any branch of the natural and clinical sciences which has a bearing upon human or animal medicine.

Long-term fellowships are normally held for one year, but may be extended to two years following a satisfactory review. These are intended for research workers **who have been engaged in research in the UK or Ireland for the past three years.** Pre-doctoral candidates may apply, but may not take up an award until their doctorate is awarded.

Short-term fellowships are available to allow more established scientists, holding substantive posts in a University in the UK or Ireland, to make short working visits of 3-6 months.

Candidates must make their own arrangements with the department in which they propose to work, and written evidence must be submitted confirming these arrangements.

Stipends according to age and experience, are within the range £8,020 to £15,193 per annum (under review). A special cost of living allowance applicable to the country in which the candidate wishes to work is given where necessary. Travelling and some incidental expenses are also provided.

Requests for application forms should include a brief curriculum vitae and an outline of the proposed research and should be addressed to: The Grants Section (Europe), The Wellcome Trust, 1 Park Square West, London NW1 4LJ.

Completed forms must be returned by 3rd April 1987.

Fellowships are also available to enable Scandinavian scientists to work in the United Kingdom. Intending applicants should contact the appropriate organisation with which the Trust has established special fellowship schemes: The Carlsberg Foundation, Denmark; The Sigrid Juselius Foundation, Finland; The Anders Jahre Foundation, Norway and the Swedish MRC, Sweden.

(2112)E

Fellowship in Cancer Epidemiology

The Preventive Medicine Institute/Strang Clinic, a cancer research institute, seeks a postdoctoral fellow to participate in ongoing studies in colon cancer using cllinical and biochemical data bases. Possible joint appointment with Memorial Sloan-Kettering Cancer Center or Cold Spring Harbor Laboratories. Prefer MD with MPH or PhD in Epidemiology, interest and experience in Oncology. Demonstrated ability to conduct research required.

Send curriculum vitae and bibliography to: Director, The Preventive Medicine Institute/Strang Clinic, 55 East 34th Street, New York, NY 10016. (NW501)E

POSTDOCTORAL FELLOWSHIP

Multidisciplinary basic cancer biology research programs at the Medical College of Virginia encompassing the areas of Biochemical, Molecular and Cellular Biology designed for both M.D.'s and Ph.D.'s and funded by a training grant from the National Cancer Institute. A broad spectrum of research areas are available including immune cell modulation (e.g., NK, LAK and signal transduction mechanisms), molecular and chemical mechanisms of carcinogenesis, gene structure/function, biochemical and molecular basis for anticancer drug action and resistance and development of biostatistical and epidemiological methods for cancer research.

Applications should include a CV ano the names of three references. Please submit to: **I. David Goldman, M.D., Program Director, Medical College of Virginia, Box 230, Richmond, VA 23298.** (NW523)E

CONFERENCES & COURSES

Royal Postgraduate Medical School
(University of London)

IMMUNOLABELLING FOR ELECTRON MICROSCOPY

19–30 October 1987

organised by the Histochemistry Unit

A two week course which will include lectures and practicals on the following topics:

Tissue preparation, fixation, processing and choice of resins; cryoultramicrotomy and freezing techniques; pre-embedding immunocytochemistry; immunoenzyme and colloidal gold techniques, colloidal gold preparation; post-embedding immunocytochemistry; peroxidase and avidin-biotin systems; multiple staining procedures including double immuno gold and protein-A methods.

Time will be available during the course for participants to carry out independent projects under supervision.

Invited speakers include;

Prof. V.M. Pickel (New York)	Immunocytochemistry of the CNS
Dr. M. Ritter (Hammersmith)	Monoclonal antibodies for immunocytochemistry
Dr. G.M. Hodges (London)	Preparation of colloidal gold
Dr. A Van Den Pol (Belgium)	Immunogold for EM
Prof. G. Coggi (Italy)	Avidin-biotin procedures
Dr. J. Leunissen (Belgium)	Immunocryoultramicrotomy

Course organisers; **Julia M. Polak, Michael J. Hedges** (Histochemistry) **Ian M. Varndell** (CRB)

Course fee:	£530 (including lunch and light refreshments but not accommodation)
Enquiries to:	Histochemistry Unit Dept Histophathology Royal Postgraduate Medical School Hammersmith Hospital Du Cane Road, London W12 0HS
Telephone:	01 740 3231
Closing date for applications:	30 June 1987

(2155)C

1987/88
INTER-UNIVERSITY
MSc COURSE ON THE
PHYSICS AND
TECHNOLOGY OF
AMORPHOUS MATERIALS

Universities of Dundee
Edinburgh — Glasgow

The one year advanced course, in this rapidly developing field, is offered jointly by the above named Universities. Course work will be given at the University of Dundee. For the project work, students will join a research group at one of the participating institutions.

Applications are invited from students with an honours degree (or its equivalent) in either Physics, Electrical Engineering, or a Materials Science. The course has been approved by the Science Research Council and Advanced Course Studentships are available for suitably qualified applicants.

Further information may be obtained from: Professor W. E. Spear, FRS, Carnegie Laboratory of Physics, The University, Dundee DD1 4HN. (2111)C

MONOCLONAL ANTIBODIES SECOND GENERATION EXPLOITATION

29th April — 1st May, 1987

UNIVERSITY OF BIRMINGHAM

An intensive course for practitioners and managers in health care or industrial production. The programme is designed to give an overall view of the subject and an insight into current thinking on production and applications.

For details contact: Dr. S. MacNeill, Co-ordinator of Continuing Education, Science Registry, University of Birmingham, B15 2TT. Tel: (021) 472 1301 ext 3668.

(2110)C

Coleg Prifysgol Cymru
Aberystwyth
The University College of Wales

M.Sc. in BIOLOGICAL ELECTRON MICROSCOPY

This one year course covers the techniques and applications of electron microscopy to biological problems. Applications are invited for places starting in October 1987.

for further details please contact:

**Dr. I. Gwynn, Department of Zoology,
University College of Wales,
ABERYSTWYTH, Wales. SY23 3DA.
Tel: 0970 3111**

(2127)C

MSc in Medical Microbiology

(University of London)

London School of Hygiene and Tropical Medicine and the Joint Medical School of University College

Advanced academic, practical and clinical training in all branches of medical microbiology for one year is provided by a full-time course by the Department of Medical Microbiology and other Departments of the School with clinical teaching by the staff of University College and the Middlesex Hospital Medical School and the Teaching Hospitals of the Schools and the Bloomsbury District Health Authority.

Specialised options for 10-12 weeks in either advanced medical bacteriology, virology, clinical microbiology, immunology, medical mycology, medical parasitology or molecular biology are available.

The MSc course is suitable for candidates qualified in medicine, dentistry, veterinary medicine, science, pharmacy and to others with approved qualifications and experience.

Successful candidates are eligible for exemption from the examination for the Primary MRCPath if other College requirements are satisfied.

Direct applications to the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, Great Britain. Application deadline: 1 May 1987.

Diploma in Medical Microbiology

A six months course of academic and practical training in selected topics, leading to the School's Diploma.

Direct applications to the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, Great Britain. Application deadline: 1 May 1987. (1389)C

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the widest international selection of jobs
in science — EVERY WEEK

Royal Postgraduate Medical School

(University of London)

MSc in EXPERIMENTAL PATHOLOGY (TOXICOLOGY)

Applications are invited for this postgraduate course from science graduates who expect to achieve, or have attained, at least a second class honours degree, and from medical and veterinary graduates.

The course, now in its tenth year, is jointly organised by the Departments of Histopathology and Clinical Pharmacology, and is attended full-time for one academic year from October. A number of Medical Research Council Advanced Studentships are available for sutiable candidates.

The course is unique in providing practical experience in both the histopathological and biochemical approaches to toxicity testing, with particular emphasis being placed upon learning modern laboratory techniques.

The course is taught primarily by RPMS clinical and scientific staff, in conjuction with invited experts from the UK: significant teaching contributions also come from the Institute of Psychiatry (Neuropathology) and Smith, Kline & French Ltd (Reproductive Toxicology).

Students prosecute a programme of original research throughout the duration of the course.

Successful graduates can expect many opportunities in histopathology, pathobiology and pharmacology both in pharmaceutical industry and in courses of study leading to a PhD. The current course is attended by 16 students.

Further details and application forms may be obtained from:
School Office (MSc), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London, W12 0HS. Telephone: 01 743 2030 ext 2242.

Closing date for the receipt of applications will be Friday 15th May 1987. (2101)C

Meeting Announcement and Call for Abstracts:

NORTH AMERICAN CYSTIC FIBROSIS CONFERENCE

Toronto, Ontario, Canada
October 15–17, 1987
Presented by:
The Canadian Cystic Fibrosis Foundation
and
The Cystic Fibrosis Foundation (United States)

A multidisciplinary conference for researchers, physicians, and allied health professionals on advances in CF research and care.

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Abstract Deadline: April 1, 1987

For further information, contact the
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6931 Arlington Road
Bethesda, MD 20814 U.S.A.
Telephone: (301) 951-4422

(NW510)C

Canadian Cystic Fibrosis Foundation
Fondation canadienne de la fibrose kystique

Cystic Fibrosis Foundation

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EMBO Practical Course on PLASMID BIOLOGY

Time: June 1–13, 1987.
Speakers: Stuart J. Austin, Gordon G. Churchward, S. Dusko Ehrlich, Saleem A. Khan, Bruce R. Levin and David Sherratt.
For further information contact: Kurt Nordström, Department of Microbiology, Uppsala University, BMC, Box 581, S-751 23 Uppsala, Sweden.
Application deadline: March 15, 1987.
Fee: 700 SEK. Lodging and meals will be provided by the organizers. The participants will have to cover their own travelling expenses.

EMBO Practical Course on METHODS FOR STUDYING CODON RECOGNITION

Time: June 17–1 July, 1987.
Speakers: J. H. Miller, Los Angeles; C. G. Kurland, Uppsala; S. Pedersen, Copenhagen; F. T. Pagel, Houston; I. Diaz, Uppsala; D. Hughes, Uppsala; F. Jörgensen, Uppsala; M. Rydén, Uppsala.
Organizers: M. Ehrenberg, Uppsala; R. Burkinghan, Paris; M. Murgola, Houston.
For further information contact: M. Ehrenberg, Dept. of Molecular Biology, Uppsala University, BMC, Box 590, S-751 24 Uppsala, Sweden.
Application deadline: April 15, 1987.
Fee: The students should be prepared to pay for their dinner meals.

FEBS Practical/Lecture course on INTRACELLULAR PROTEIN TRANSPORT

Time: June 22–29, 1987.
Speakers: W. Balch, A. Colman, C. Gahmberg, H. Garoff, H. Glauman, K. Howell, M. Makarow, R. Pettersson, S. Kvist, A. Rambourg, K. Simons.
Organizers: E. Fries, R. Persson and T. Berg.
For further information contact: Dr. Erik Fries, Dept. Med. Phys. Chem., BMC, Box 575, S-751 23 Uppsala, Sweden. Telelephone: 18-17 41 99, Telefax 18-15 17 59.
Application deadline: March 15, 1987.
Fee: 1800 SEK. Lodging and meals will be provided by the organizers. The participants will have to cover their own travelling expenses. A limited number of fellowships will be available — please indicate your interest on the application form.

BIOLOGY OF THE MACROPHAGE

Time: August 30–September 4, 1987.
Organizers: Professor Rolf Seljelid, Tromsö, and Dr. Bård Smedsröd, Uppsala. International speakers will be recruited. The participants are encouraged to discuss their own research problems.
For further information contact: Professor Torvard C. Laurent, Department of Medical and Physiological Chemistry, BMC, Box 575, S-751 23 Uppsala, Sweden. Telephone: 18-17 41 55.
Application deadline: June 15, 1987.
Fee: The course is sponsored by Sätra Spa. There is no fee.

EMBO Practical Course on PROTEIN CRYSTALLOGRAPHY

Time: September 7–18, 1987.
For further information contact: Dr. T. A. Jones, at the Department of Molecular Biology, Uppsala University, BMC, Box 590, 2-751 24 Uppsala, Sweden.
Application deadline: April 15, 1987. Applications, including a C.V. of current research should be sent to Dr. T. A. Jones.
Fee: 500 SEK.

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Further information and application forms from: **Dr. D.E. KIDD, Dept. of Physics and Applied Physics, University of Strathclyde, 107 Rottenrow, Glasgow G4 0NG, Scotland.**

(Closing date for applications 1st May 1987) (2158)C

SEMINARS & SYMPOSIA

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DISCUSSION MEETING
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28 and 29 May 1987, organized by Professor C.J. Leaver, F.R.S., and Dr D.M. Lonsdale

Speakers include: L. Grivell, M. Gray, D.M. Lonsdale, P. Slonomski, R. Butow, A. Tzagoloff, C.S. Levings, D.R. Pring, C.J. Leaver, J. Laughnan, T. Fox and G. Schatz.

Registration details (no fee for attendance): **Miss C.A. Johnson (ref. DMN/CAJ), the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG (Tel: 01-839 5561 ext 277. Telex 917876).**

(2107)M

MOLECULAR BIOLOGY OF CANCER

at the

IX Meeting of The European Association for Cancer Research (EACR-IX)

Helsinki, Finland, 1-3 June 1987

Plenary lectures on:
Molecular Biology of Oncogenes
Molecular Basis of Carcinogenesis
Molecular Epidemiology of Cancer

Symposia on:
Cellular Oncogenes and Transforming Proteins
Chromosomes, Oncogenes and Cancer Genetics
Viral Carcinogenesis
Growth Factors, Hormones and Cancer
Cancer and Cell Differentiation
Tumor Promotion and Progression
Invasion and Metastasis
Host Defence
Molecular Basis of Cancer Chemotherapy
Monitoring of Carcinogenic Exposure
Susceptibility to Cancer
Epidemiological Approaches to Carcinogenesis

Invited speakers will include:
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A. Balmain
M. Barbacid
H. Bartsch
E. Canaani
P. Comoglio
C. Croce
K. Danø
N. Day
G. Evan
T. Graf
G. Grosveld
H. zur Hausen
C–H. Heldin
F. Kadlubar
G. Klein
P. Lohman
J–P. Mach
V. Moolenaar
H. Moses
K. Nilsson
M. Oren
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Finland, phone 358-0-611050

(W3828)M

WORKSHOPS

GULBENKIAN INSTITUTE OF SCIENCE, PORTUGAL

In 1987 the following workshop courses will be offered:

Regulation of Gene Expression in Eukaryotic Cells. 26 April - 2 May, R. Planta (Amsterdam), W. Keller (Heidelberg), R Burdon (Glasgow), A. Sentenac (Gif-sur-Yvette), C. Rodrigues-Pousada (Oeiras).

Isolation, Identification and Maintenance of Yeasts. 10 - 28 August, J. P. van der Walt (Pretoria), M. Th. Smith (Delft), B. Kirsop (Norwich), T. Boekhout (Baarn), N. van Uden (Oeiras).

Membrane Receptors, Transducing Mechanisms and Neurotransmitter Release. 7 - 18 September, T. W. Stone (London), P. J. Marangos (Bethesda), K. H. Jacobs (Heidelberg), J. A. Ribeiro (Oeiras).

Information and Registration Forms: Professor N. van Uden, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal.

(W3681)V

ANNOUNCEMENT

III International Workshop on Carcinogenesis Studies in Human Tissues and Cells

Elsinore, Denmark, October 5–8, 1987

Sponsored by the Danish Cancer Society

This workshop will focus on the recent developments concerning molecular and cellular mechanisms of carcinogenesis. The meeting will cover the following subject areas:

Growth Factors and Differentiation, Tumor Promotion, Tumor Biology and Metastasis, Transformation of Human Cells, Host Factors and Biochemical Epidemiology, Human Tumor Viruses, Somatic Cells and Molecular Genetics.

Among the invited speakers are:

H. Autrup, A. Balmain, C Bertzholz, F. Blasi, P. Cerutti, D. Crawford, D. S. Davies, W. Franke, N. Fusenig, R. Gallo, C. Harris, V. Heyningen, J. Hoeijmakers, T. Kakunaga, T. Krontiris, J. Lechner, L. Liotta, P. Parker, L. Phillipson, C. D. Stiles, E. Stanbridge, H. zur Hausen, U Wewer.

The meeting will have limited participation, and the participants will be selected on the basis of the submitted abstracts.

Further details and abstract forms:

Danish Cancer Society
Attn. Dr. H. I. Nielsen
Rosenvængets Hovedvej 35
DK-2100 Copenhagen 0
Denmark
Phone: +45-1-268866, ext. 450

Abstracts are due no later than May 15, 1987. The selected participants will be informed by June 30, 1987.

(W3824)V

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independent foundation, created in 1971, to promote scientific research in all disciplines which contribute towards improving human health.
This aim is pursued by means of congresses, courses, seminars and specialized studies.

Some of the international meetings scheduled for 1987 are:

Herpes and Papilloma Viruses
Milan, Italy / March 26-27
Scientific Organization: F. De Palo (I), F. Rilke (I) and H. Zur Hausen (D)
IV Pan American Congress of Andrology
São Paulo, Brazil / May 4-6
Scientific Organization: A. Negro-Vilar (USA) and M. P. De Castro (BZ)
Inhibin - Non-Steroidal Regulation of Follicle Stimulating Hormone Secretion
Tokyo, Japan / May 21-22
Scientific Organization: H. Burger (Aus) and M. Igarashi (J)
Development and Function of the Reproductive Organs
Turku, Finland / June 10-12
Scientific Organization: M. Parvinen (SF)
IV Colloquium of the European Pineal Study Group
Modena, Italy / August 31 - September 4
Scientific Organization: G.P. Trentini (I), A. Oksche (D) and P. Pevet (F)
Cell-to-Cell Communication in Endocrinology
Florence, Italy / October 8-9
Scientific Organization: L. Martini (I), M. Serio (I) and C.W. Bardin (USA)
Differentiation Therapy for Cancer
Tucker's Town, Bermuda / October 23-25
Scientific Organization: G.B. Rossi (I), F. Takaku (J) and S. Waxman (USA)

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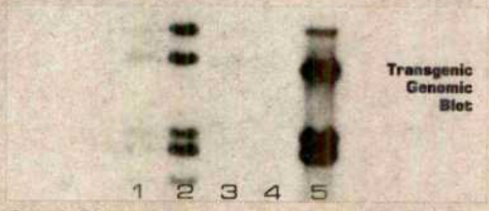

Genomic DNA was digested to completion with Bam HI, then electrophoresed on a 0.8% agarose gel. The Southern blot was screened with a 32P-labeled, nick-translated DNA probe. Lanes 1 and 2: DNA correlating to 1 and 5 haploid genomic equivalents of DNA, respectively. Lanes 3 and 4: Genomic DNA from negative controls not containing integrated DNA. Lane 5: Genomic DNA from positive transgenic containing greater than 5 copies of the integrated gene.

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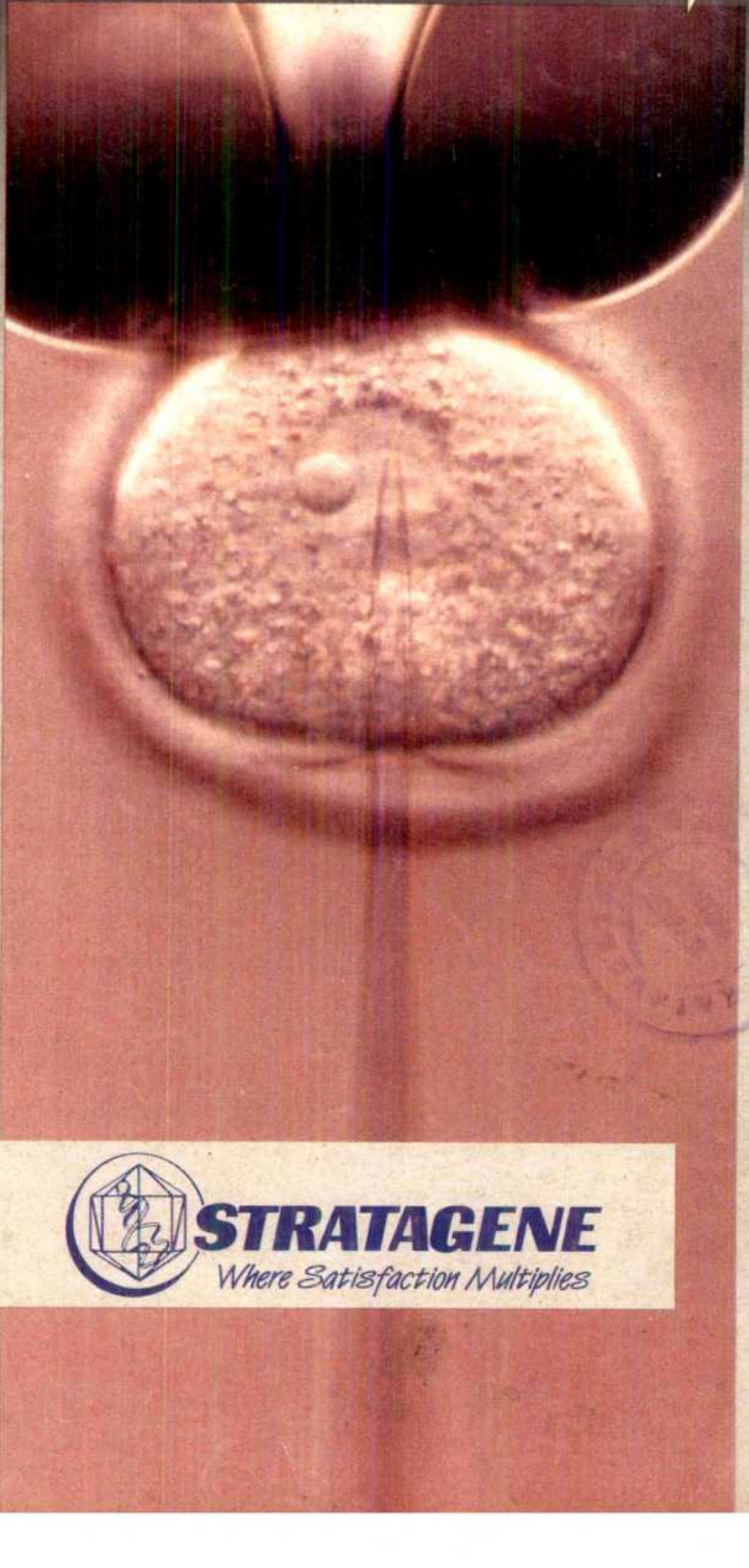

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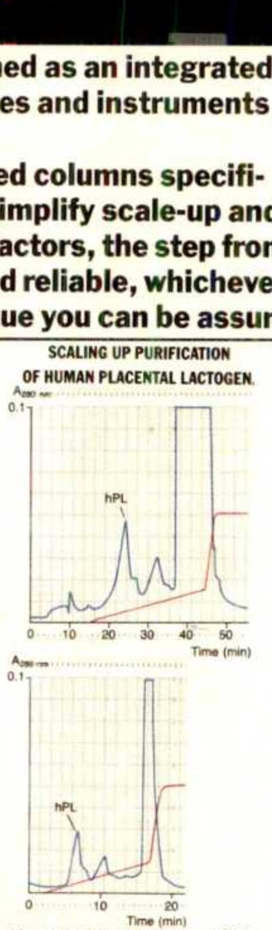

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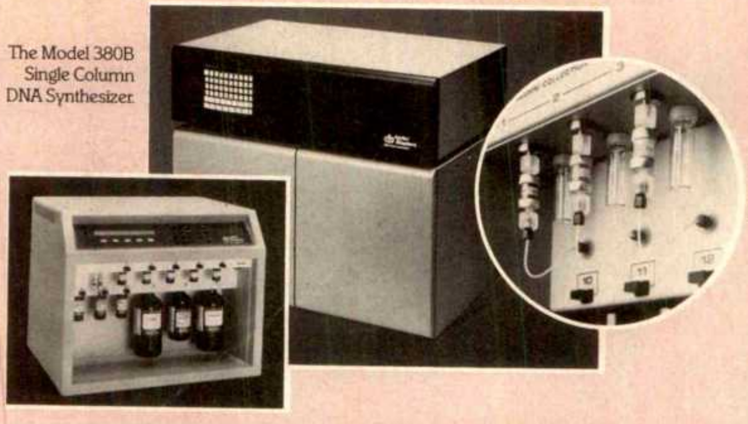

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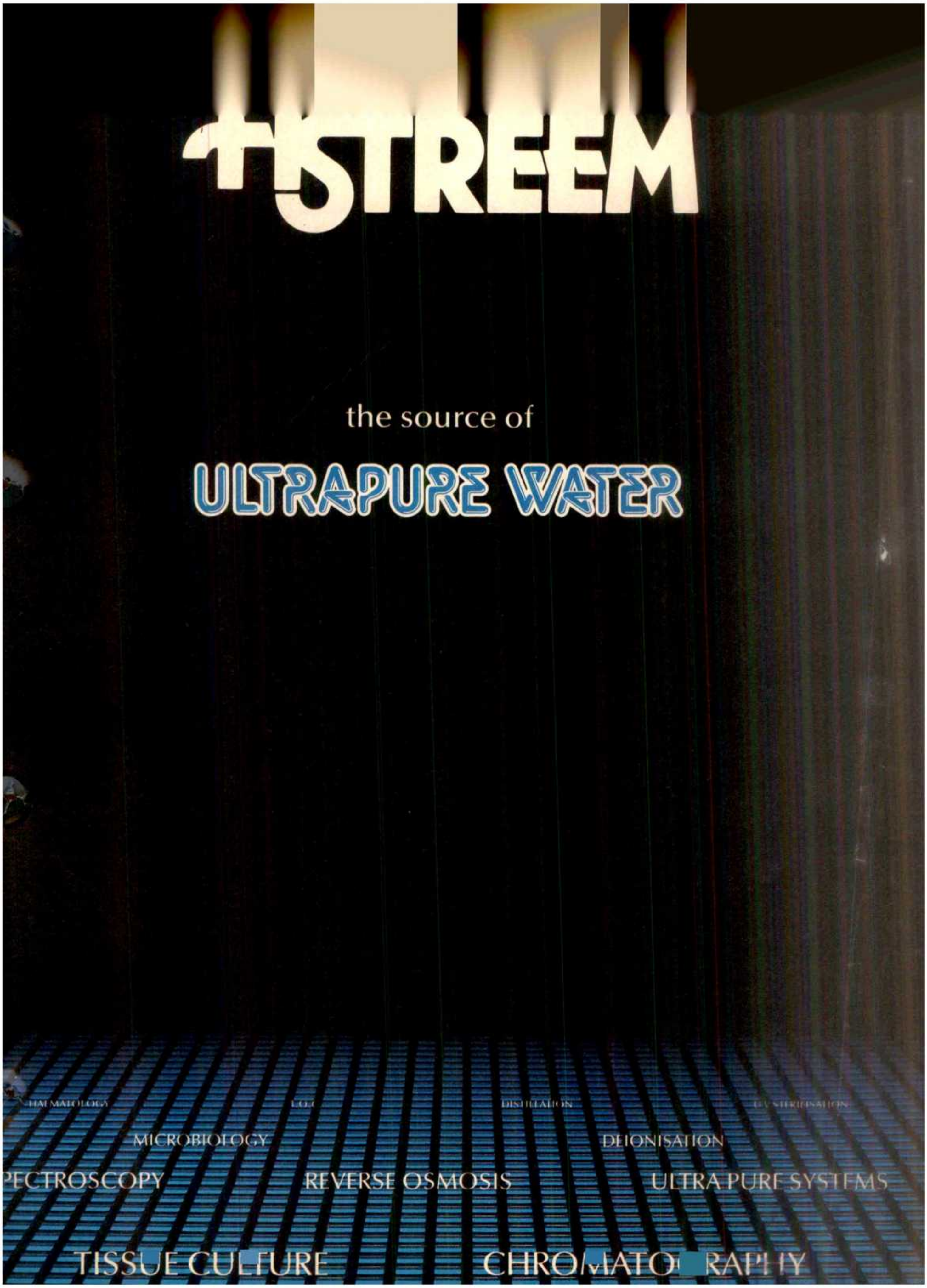

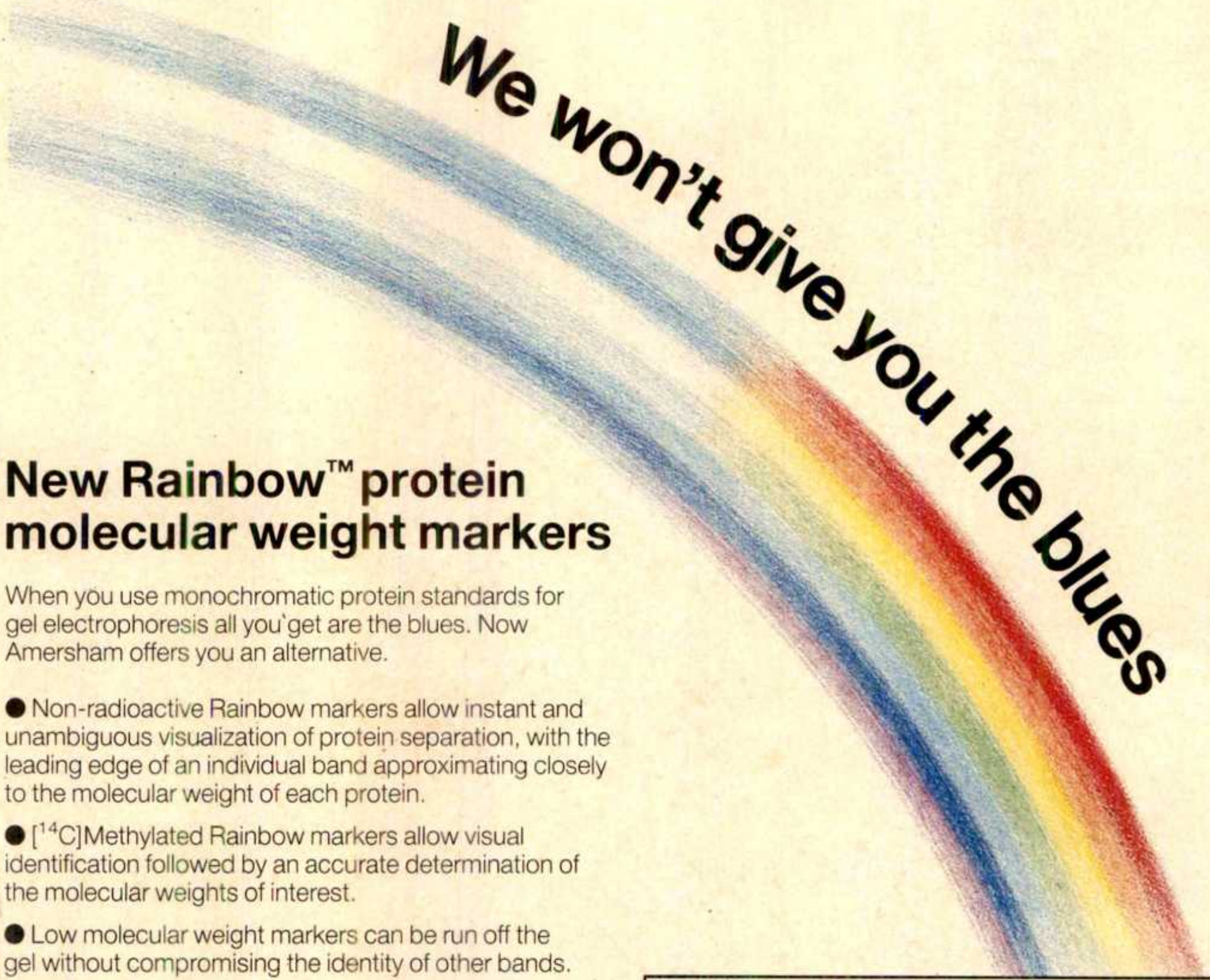
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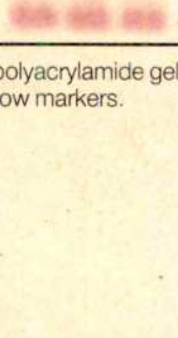

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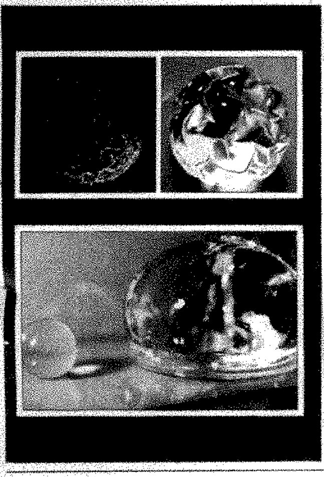


nature

26 February 1987
Volume 325
Issue no. 6107

When a spherical acrylamide gel swells in water into a large sphere, a regular pattern appears on the surface. The unit size of the pattern doubles with time becoming comparable to the gel radius, and finally disappears. The time of immersion in water for each picture is; top left, 2h, top right, 1 day, bottom, 7 days. See pages 796–798.

THIS WEEK

■ First gene for psychiatric illness

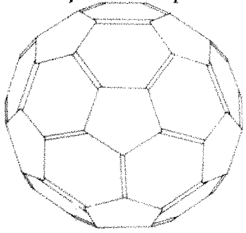
. . . pages 756,783,805 and 806

Manic depressive illness is inherited in the Old Order Amish community in southern Pennsylvania. The gene responsible has been located near the short end of chromosome 11, close to the gene for tyrosine hydroxylase which plays a part in the formation of dopamine. But genes on different chromosomes cause the disease in other families.

■ Ring currents

. . . pages 760 and 792

Buckminsterfullerene, a carbon cluster suggested to be the first example of a spheroidal 'aromatic' molecule, has magnetic properties unlike those of any molecule yet encountered.



■ Drugs against AIDS

. . . page 773

The variety of therapeutic strategies suggested by the life cycle of the AIDS retrovirus is reviewed. The positive clinical trials for AZT and studies with a new drug that may be more effective than AZT in disrupting viral replication are highlighted.

■ AIDS vaccine

. . . page 765

The harmful effects of some animal vaccines against lentiviruses suggest that there may be dangers in AIDS vaccines even if they do not contain the human immunodeficiency virus itself.

■ Giant pandas

. . . page 758

Plans presented jointly by the World-Wide Fund for Nature and the Chinese government offer some hope for the conservation of still declining panda populations.

■ Lipid tracers

. . . pages 761 and 800

Plant lipids present in organic aerosols are combined with isentropic trajectory calculations to trace source regions and transport paths of winds in the Southern Hemisphere.

■ Red-giant star

. . . page 787

A stellar outburst from what appears to be a red-giant star is a rare glimpse of the late stages in star evolution. Two luminous arcs are shells of gas expanding outwards from the central star which is obscured by dust.

■ Redesigning the fruitfly

. . . page 816

A genetic manipulation experiment that results in the replacement of antennae of *Drosophila* by legs adds weight to current ideas on the genetic control of body plan.

OPINION

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746 US university risks

NEWS

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SCIENTIFIC CORRESPONDENCE

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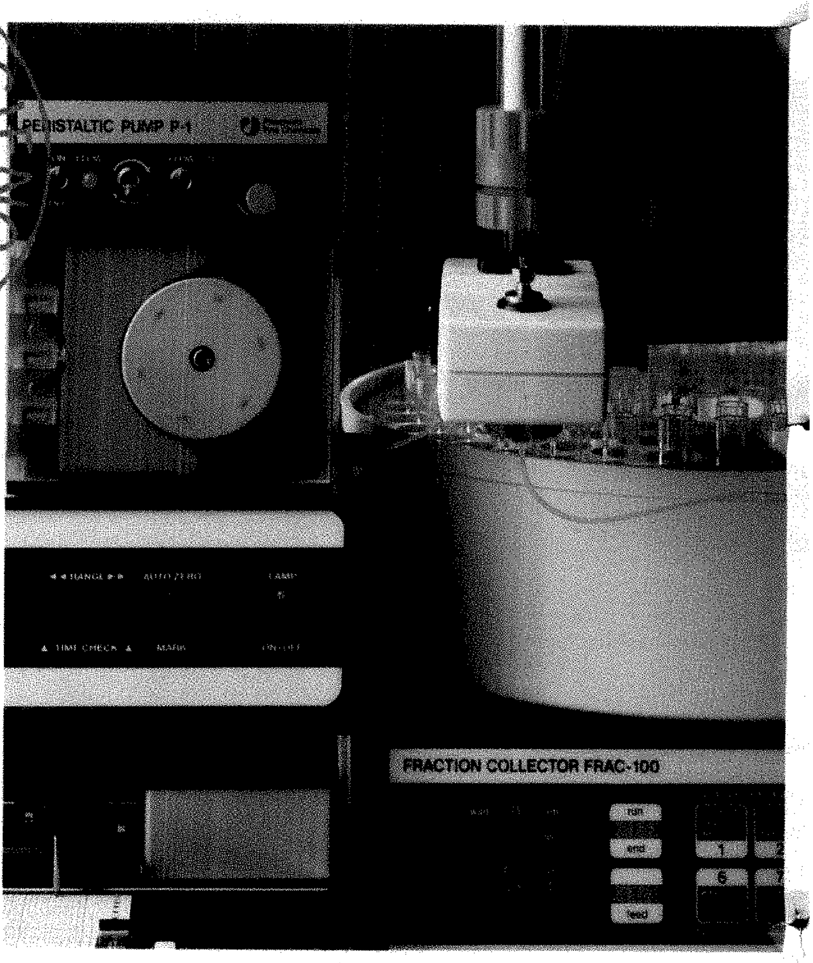

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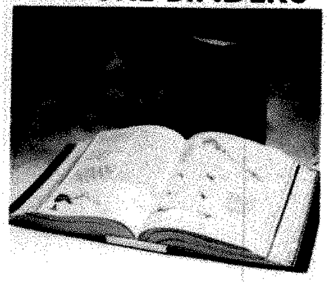

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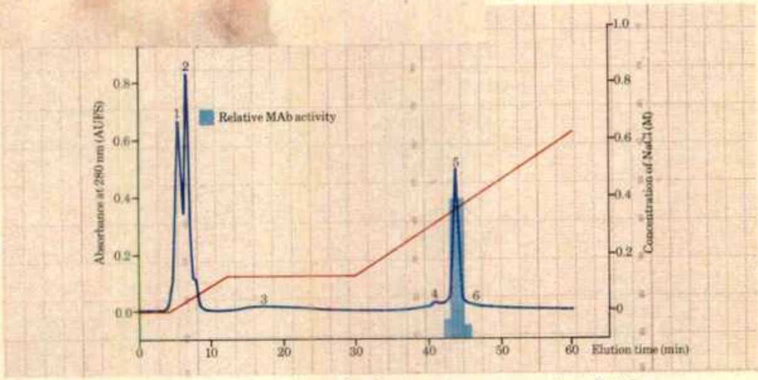

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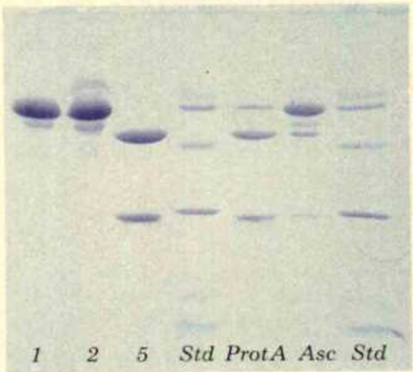

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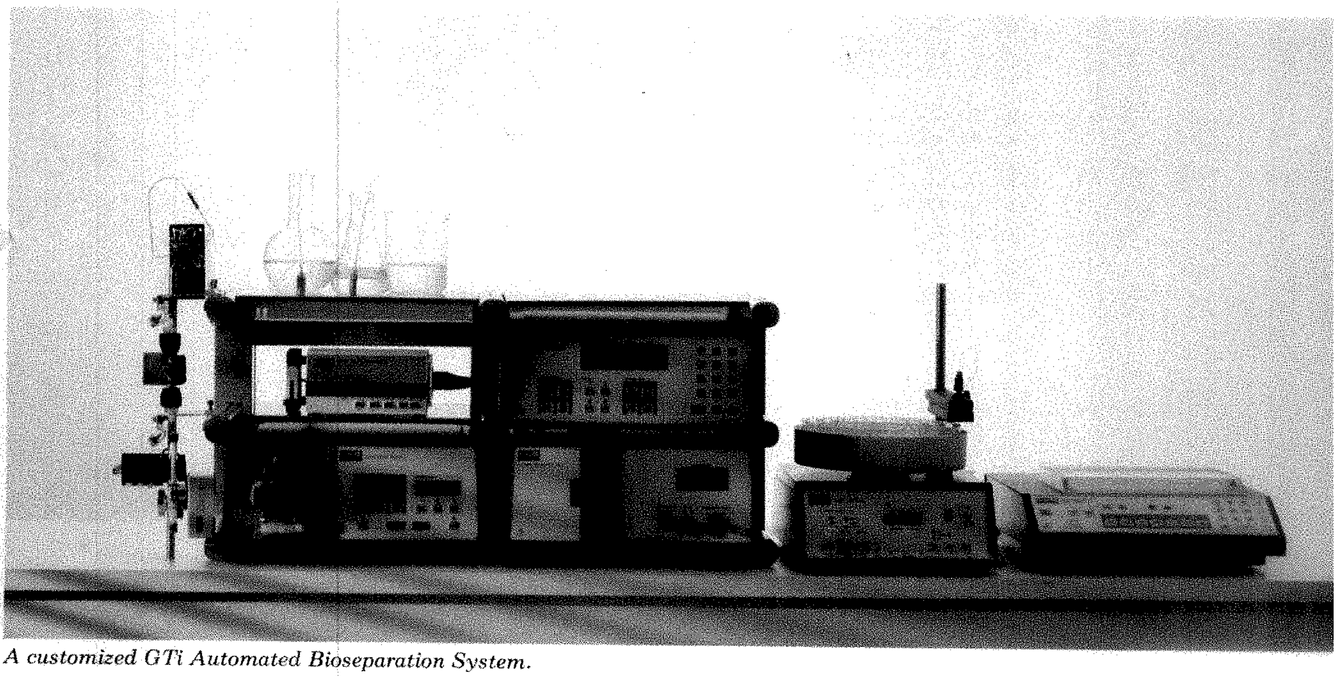

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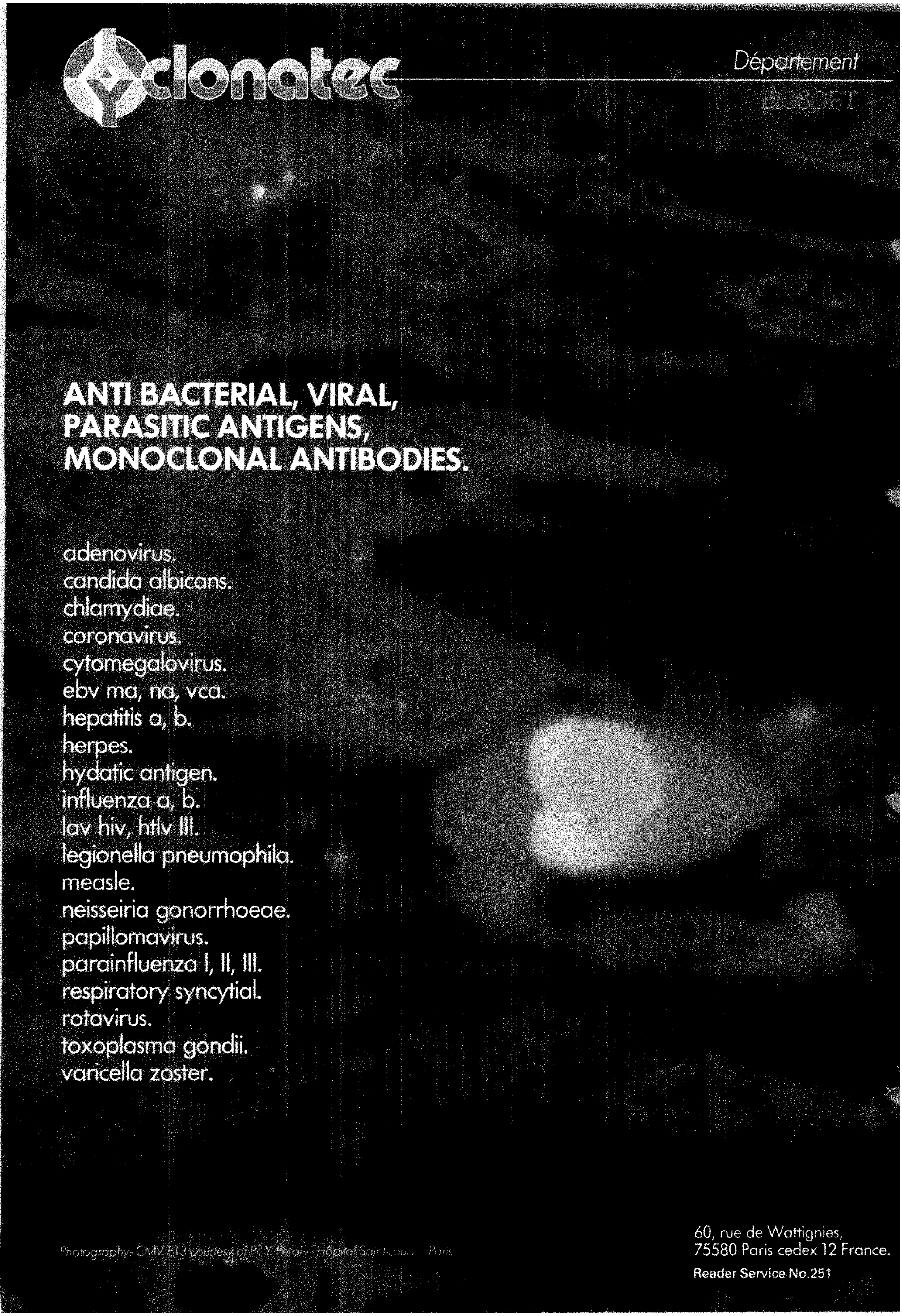

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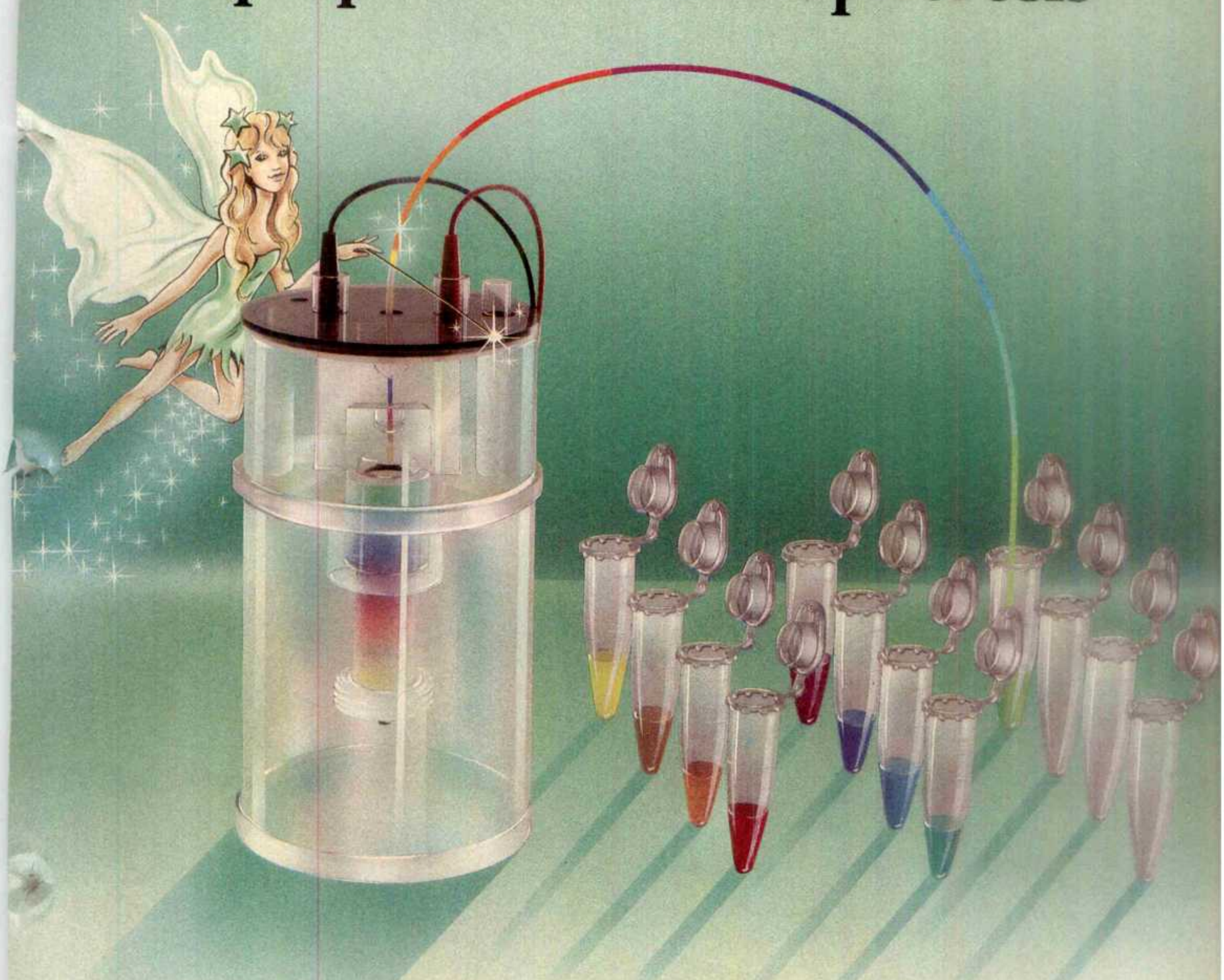

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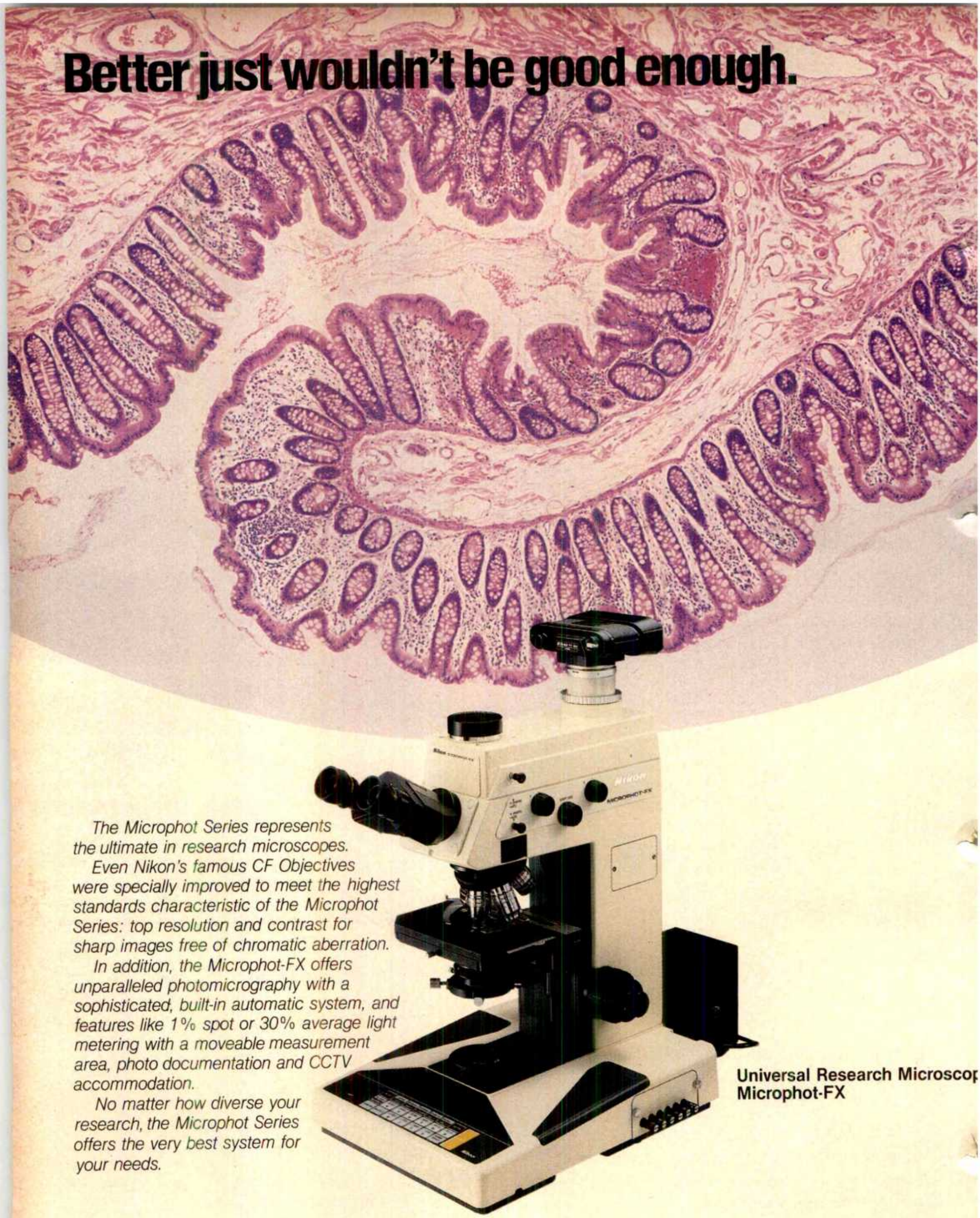

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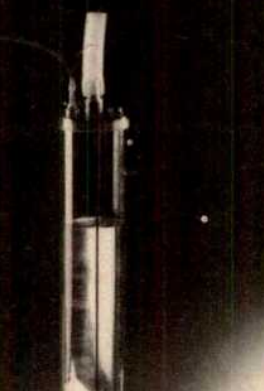

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human T-cell T8 (CD8), clone FK 18
human T-cell T9/Transferrin receptor, clone 66-IG10
human HNK-1, clone 6.13.19.1
human Monocyte/Granulocyte antigen (CD11), clone FK24
human Granulocytes (CDw15), clone FK32
HLA-D, clone 7.510.1
HLA-DQw1 related determinant, clone II B3
HLA-DRw52 related determinant clone 7.3.19.1
*human T-cell receptor, clone WT31
*human T-cell T3 (CD3), clone WT 32
*human T-cell T8 (CD8), clone WT 82
*human CR3 (CD11b), clone Bear-1
*human LFA-1 (CD11a), clone SPV-L7
*human Myeloma antigen, clone Gepl-1

HORMONES

human Chorionic Gonadotropin, (hCG); clone 2092
human Follicle Stimulating hormone, (FSH), clone 2093

INTERMEDIATE FILAMENTS

human Desmin, clone 33
human Glia Fibrillary Acidic Protein, clone 6F2
human Keratin, clone 80
human Neurofilament, clone 2F11
human Vimentin, clone 9
*human Keratin 18, clone M9

CYTOSKELETON ANTIGENS

actin, (rabbit muscle), clone SA1C1
tubulin, (rat), clone 1G9

SERUM PROTEINS

human Kappa, clone 2B7
human Lambda, clone 48
human Thyroglobulin, clo
human IgM, clone B8904

HISTOLOGICAL A

human Endothelium (bloo
human Endothelium, clon
*human Neurofibrillary Tan

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*human Placental Alkaline
*Gamma Enolase, clone M

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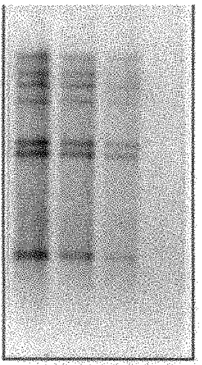

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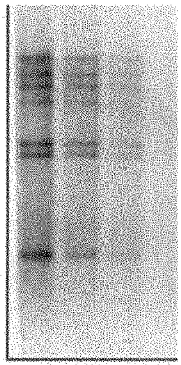

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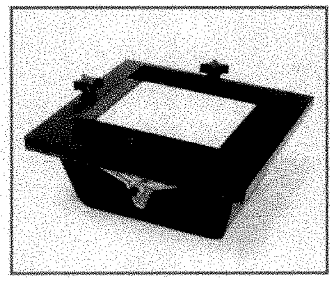

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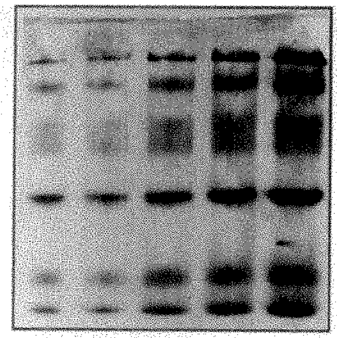

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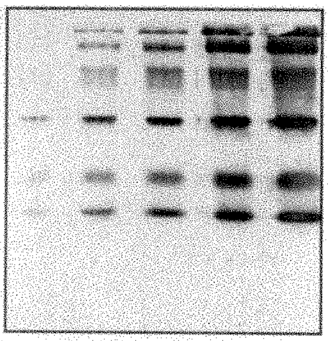

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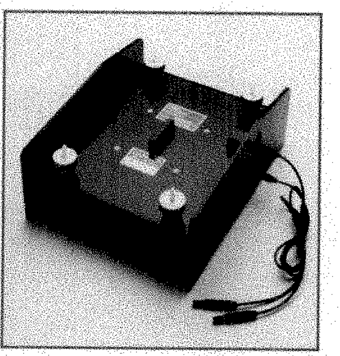

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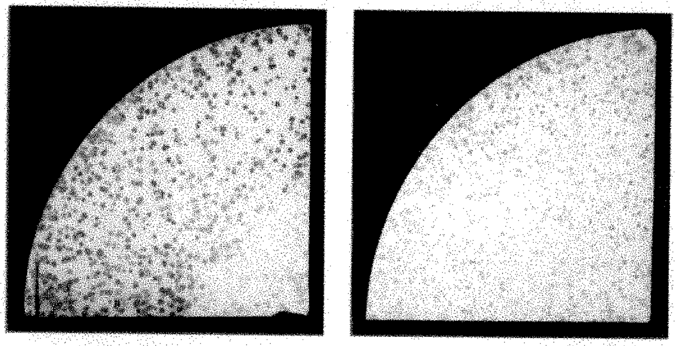
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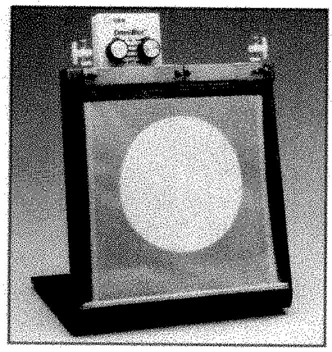

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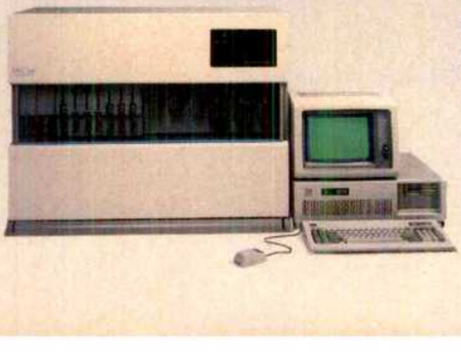

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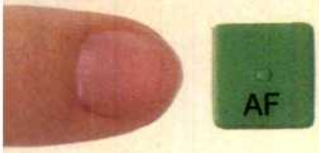

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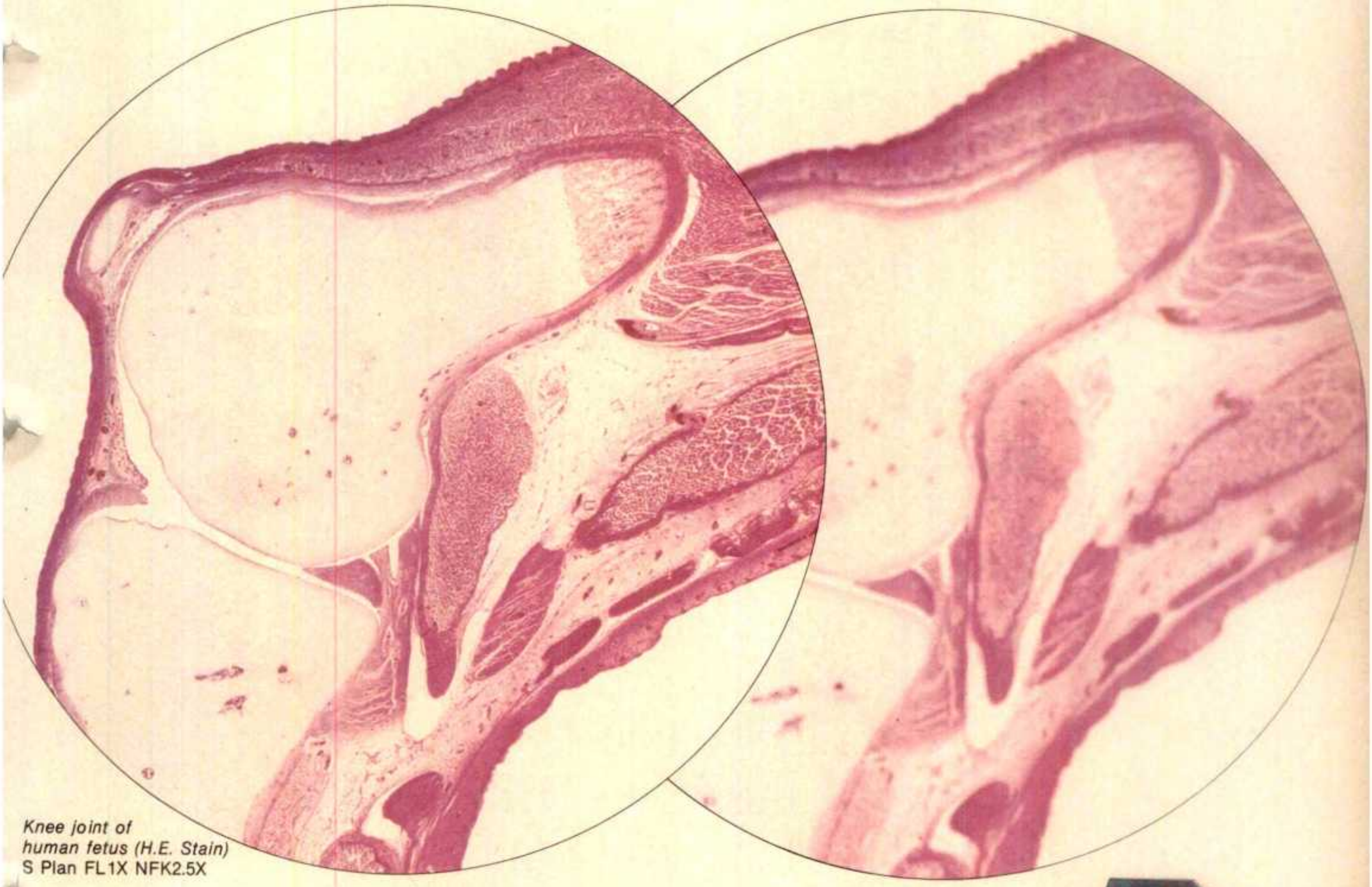

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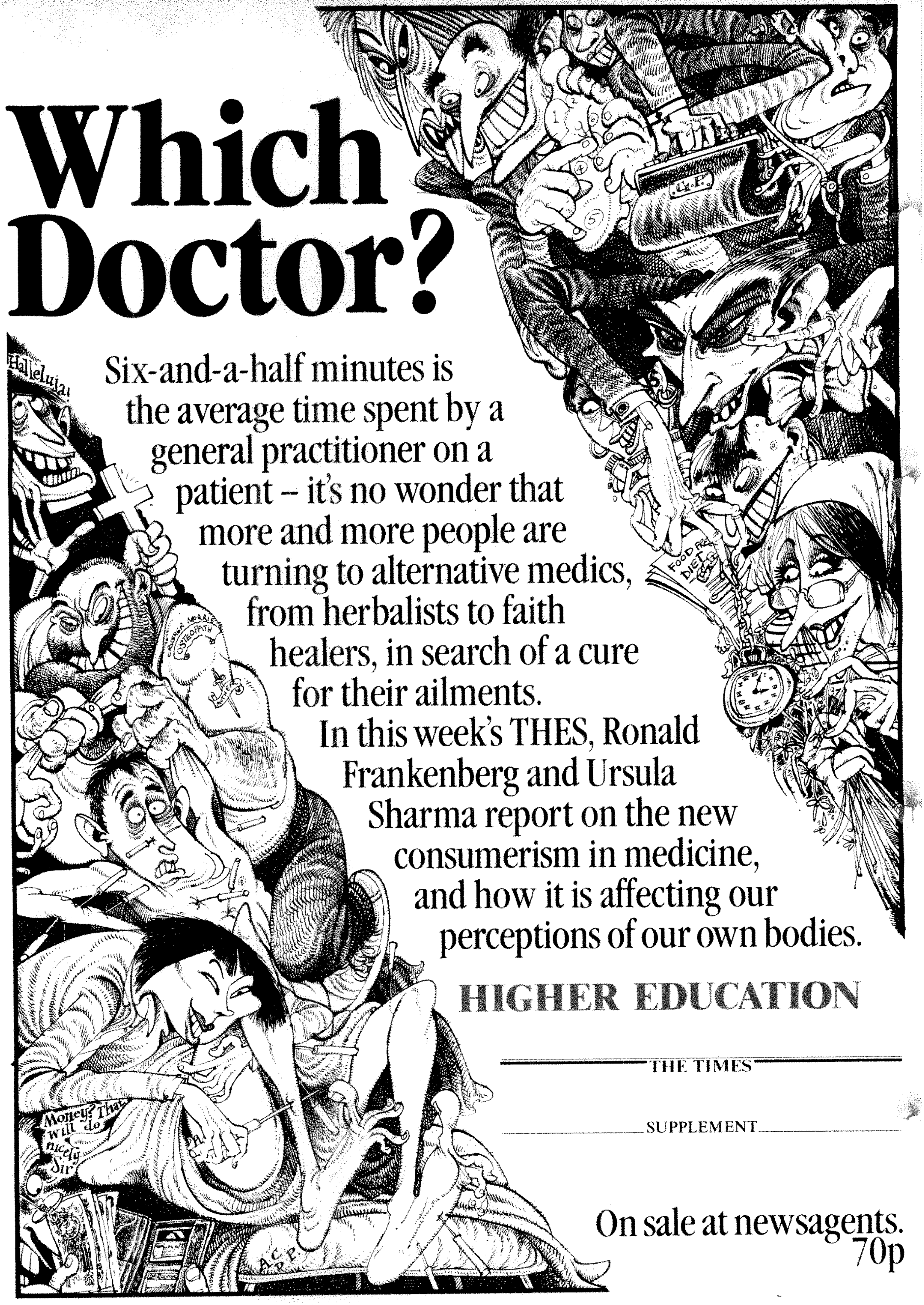

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In this week's THES, Ronald Frankenberg and Ursula Sharma report on the new consumerism in medicine, and how it is affecting our perceptions of our own bodies.

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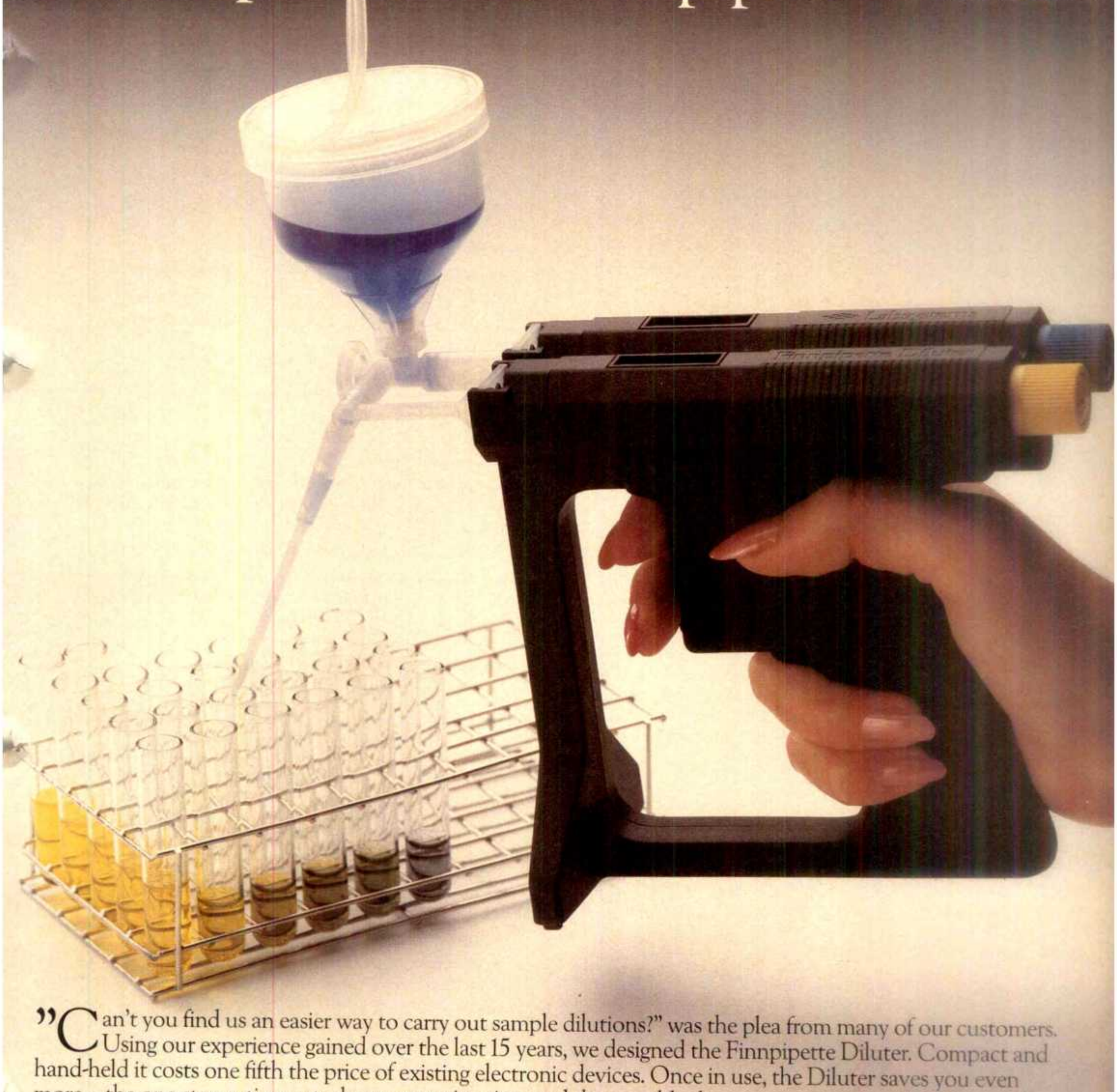

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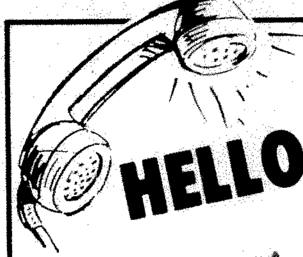

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Who wants the US space station?

The US plan to put a space station into an orbit about the Earth has little to commend it except that it will show that the United States is still in space. But there are better and safer ways of leading.

The motto that it never rains without pouring must seem apt to the embattled administration of the United States. Very little seems to be going right. That is why, in a week in which the reputation of the presidency is likely to be further damaged by Senator John Tower's report on the sale of munitions to Iran, it may seem unkind to be rubbing salt in another of the administration's self-inflicted wounds. Yet, on the same calculation, this is when the astringent is most likely to be effective. And that is why the United States should now bite on another bullet and abandon its plan to build a space station in an orbit about the Earth.

When first put forward, three years ago, the plan seemed to some a natural way of building on the promised success of the space shuttle, a remarkable technical achievement in spite of last year's tragic accident. Given a means of carrying people and equipment into orbit for a week or so, why not use the same device to establish something more permanent? The outcome was an enterprise, jointly with Canada, the European Space Agency and Japan, to install a space station in 1994. Now it is inevitable that completion must be delayed, not so much because of last year's accident but because of technical difficulties that have increased the estimated cost of the original design from $8,000 million to more than $14,000 million. During the next six months, the US National Aeronautics and Space Administration (NASA) will be seeking some kind of compromise with a budget-conscious Congress, leaving its partners in limbo the while. Both parties to that dialogue should appreciate that it would be better to cancel the project now, before it becomes an albatross around NASA's neck.

The least important facet of the argument that lies ahead is that which has received the most attention in the past few weeks, the possibility that the space station might provide house-room for experiments commissioned by the US military. NASA rightly says that its partners have known from the outset that the US Department of Defense might wish to share the space station with civilian users (some of them commercial and thus as obsessed with secrecy as the military). Present anxiety on this question, most acute in Europe, is largely the result of poor timing. The Pentagon's reasonable request, towards the end of last year, for a say on the agreements being negotiated with potential partners coincided with its push for a decision to deploy some elements of the Strategic Defense Initiative (SDI) long before 1993, the date foreseen when SDI was launched on an astonished world. Logically, there is no connection between these developments, but NASA and the Pentagon have been slow to see the potential connection, the fear of many in Europe that an accommodation on military uses for the space station may seem to imply approval for the early deployment of SDI. The difficulty would not have arisen if the diplomats had done their paperwork more quickly. The next few weeks should tell whether it can be chased away. Failure would weigh heavily with Congress during the budget process.

Other reasons for cancelling the space station bear more directly on the plan itself. One difficulty is that the capacity of the shuttle to carry hardware into orbit will be much less than foreseen three years ago; even when the lost craft is replaced, the frequency of flights will be more like one a month than one a fortnight. What will happen to the payloads crowded out by the components of the space station? Another problem is the prospect that this project, like the shuttle programme after the first flush of enthusiasm, will have to be run on a shoestring. Although it is too soon to know whether the new US Congress will face up to its fiscal responsibilities to bring the budget deficit within limits, it is more than likely that a necessarily uncertain project such as the space station costing, even now nearly as much as the Apollo programme of the 1960s will, at some point in the next seven years, be forced to rub along with funds insufficient to guarantee success. And what would happen if the linked evils of the US budget and trade deficits should bring about the sharp recession about which economists speculate more openly every day?

Strategy

Prudence, and NASA's painful experience of being so committed to the shuttle at a time of budgetary restraint that it had to leave aside many smaller useful projects, argue for a less risky strategy. Neither the administration nor the US Congress seems to realize that the most curious feature of their strategy in space has been its discontinuity. Having reached the Moon, NASA built the shuttle. Having, with the Pioneer and Voyager space craft, shown how to explore the distant reaches of the Solar System, NASA has no plans to follow through. Having put two superb X-ray telescopes in orbit, NASA seems willing to leave to others the design of their successors. Meanwhile, the agency can only receive in embarrassed silence the repeated reasoned arguments from official and unofficial advisory groups that urge this or that good project in the exploration of space. (Another list of unrequitable wishes from NASA's own science advisory committee is due out this week.) The result has been the alienation of a large part of the scientific community.

Nor can NASA claim that it has no more money for basic science because it is pursuing the applications of satellite technology instead. Here, too, NASA is a feckless innovator. Having pioneered Earth observation satellites, it must contemplate the superior performance of the French instrument called SPOT. The hope that the US Navy would assume (from the National Oceanographic and Atmospheric Agency) responsibility for the sea-surface observation satellites seems to have been thwarted by the Navy's cash problems, but is it really the US government's intention that such projects should be the vicarious casualties of random budget shortages? And while NASA may be right to have decided (in the budget for the next fiscal year) that the project to build an advanced telecommunications satellite can be safely left to private industry, there is now no knowing when that will be done. In applications as in the basic science programme, NASA cuts the figure of a fickle will-o'-the-wisp, trying one thing and then another. The agency's conventional wisdom is that it has no choice, for Congress would 'never' agree to spend much more than $2,000 million on these unspectacular tasks. The statement would be more telling if NASA had ever sought to persuade people otherwise.

It is thus not surprising that US taxpayers and even NASA officials are beginning to ask how "leadership" in space can be assured, even recovered. As usually asked, the question is vacuous. Leadership is taken as a synonym for 'in the lead' or 'first'. On this philosophically bankrupt basis, last year's shuttle accident is bound to seem not merely a tragedy but a threat to leadership. The truth is that a better synonym for leadership in technological connections is expertness, the capacity to carry out difficult tasks with ease and confidence. Now that being ubiquitously first (on the Moon, in a space station or even on Mars) is financially and technically impossible, not to say philosophically pointless, should not NASA aim at the more durable leadership that could come from doing excellently what it does well already? It would be a less spectacular programme, but the United States might welcome a respite from sensation. □

Ivan Boesky's friends

The insider-trading scandal seems unstoppable, but there are some things that could be done.

The great worldwide financial scandal goes from bad to worse, but governments and financial communities appear mesmerized by what has overtaken them, and to be incapable of response. Last week, Mr Dennis Levine, the insider-trader who first blew the whistle on Mr Ivan Boesky, was sentenced by a New York court to four parallel terms of two years' imprisonment. A week earlier, an even bigger fish, Mr Martin A. Siegal, had pleaded guilty to two felony charges and had declared that he is cooperating with the authorities (which no doubt signifies that there are more surprises to come). By all accounts, the indictment of Siegal explains that a courier working for Boesky had at one stage handed him by prearrangment in a hotel lobby a suitcase containing $150,000 in notes; the money was used in succeeding years to pay minor household bills. A few days earlier, a senior member of the stockbroking firm of Goldman, Sachs and Co. was arrested together with two others from Siegal's old firm, Kidder Peabody (but Goldman, Sachs says it will pay the cost of defending its Mr Robert M. Freeman).

These events would be fascinating if they were not also tragic and frightening. Commenting on the Siegal case last week, the *Wall Street Journal* says that it shows that insider-trading has become "systemic" in New York. That is frightening because it gives the lie to the belief, much cultivated by the self-interested financial communities, that the markets are efficient and are thus reliable ways of deciding the fate of enterprises of all kinds, from established industries to struggling new ventures. To the extent that the galloping scandal (and its Guinness offshoot in London) shows that it is possible to manipulate the price of publicly traded stock, even at some cost, it is no longer possible to believe that what happens on the markets equates with common sense. Worse still, the manipulators have been driven by the attraction of personal gain, in Boesky's case on a scale that enabled him to pay a fine of $100 million and still have some left over; in doing so, they have been stealing from other people, often those ordinary mortals who put their savings in pension funds and other financial securities.

What can be done? It remains to be seen what are the effects of the gaol sentences now being handed out, but of necessity they will not be permanent. In the US Congress, Senator William Proxmire has followed the British Labour Party in suggesting tighter regulation of the takeover process (which attends to the substrate of corruption, not its cause). In both places (and elsewhere) it would make better sense if it were a legal requirement that the true owners of all traded stocks should be instantaneously and publicly known. Then at least there would be a chance that the professionals would catch each other out. And why should not the people who work in the financial markets be forbidden from owning stock on their own account? If it should be said that people would not do this job if deprived of the opportunity for making a little extra on the side, that would be a proof that insider-trading is indeed systemic. □

US university risks

British universities have lost their political friends. Will US universities follow suit?

Could the university system in the United States be running headlong into the problems that have overtaken British universities in the past decade? On the face of things, that is impossible. Unlike British universities, whose costs are mostly met directly from the public purse, universities in the United States are either private institutions whose autonomy is beyond dispute or are supported by state governments jealous of their institutions' achievements and conscious of their economic importance. That is the theory. But US universities, and especially the private institutions, may now be courting just the danger that, ten years ago, marked the onset of the British government's beastliness to its universities. The US universities, or some of them, run the risk of losing their political support, ironically even when the economic value of the skills that universities can engender is more keenly appreciated than ever.

The underlying issue, first in Britain and now in the United States, is public accountability. In Britain in 1967, Mrs Shirley Williams, then Secretary of State for Education and Science in a Labour government, asked the universities two pointed questions: how would they respond to the demographic prospect of falling enrolments, and what suggestions did they have for helping a financially beleaguered government contain the cost of student maintenance? The replies — that shrinking age-groups do not imply shrinking universities, and that there is a case (in British circumstances) for paying maintenance costs to all, are reasonable points of view — but took no account of the administrative reasons why even a sympathetic minister should have been compelled to raise these awkward questions.

Nobody would suggest that present circumstances in the United States are the same. Mr William J. Bennett, the Secretary of Education, makes very few noises of the kind that academics like to hear (see p.751). Moreover, federal aid to higher education is a small percentage of the total cost of higher education, and shrinking. Yet Bennett, like Williams, is asking questions that universities would be well advised to answer patiently. Is it, for example, true that tuition fees have increased on the average by 58 per cent in the past five years, twice as quickly as inflation, and if so why? If, as some institutions will be tempted to say, the explanation is that higher tuition fees are needed to meet the disproportionately increased need for student aid, there will be many who ask what business it is of universities to operate a redistributive taxation system. And is it true that average university salaries have kept pace with inflation in the past decade, when one of the most striking social changes in the United States has been a relative decline of individual, as distinct from family, incomes (as women go out to work)? Universities will say that to pursue excellence they must compete for what talent there is, but there are many who will protest that the bidding process differs from that among baseball teams in that the costs fall on students who have no choice but to pay up.

What US universities may not have understood clearly is that Mr Bennett's questions, even if misconceived, may anger those who hear them answered superficially, disdainfully or casuistically. What the British experience has shown is that it is easier to provoke a hostile animus than to mollify it, especially among politicians. The moral is that Bennett should be given a reasoned and comprehensive answer to the questions he keeps flinging at the system, but also a more constructive account of what needs to be done to keep the system healthy. The weakest point in his argument is the assumption that even students from the poorest families will become so aware of the benefits of higher education that they will shoulder the burdens of commercially priced loans to win the rewards. It is unlikely that the United States is any better able than Britain to risk the loss of talent provoked in that way. □

World trade war looms over microchip accord

- USA demands that Japan fulfil trade pact
- EEC calls for ruling on 'illegal' trade plan

London and Washington

A MAJOR trade row is about to erupt, embroiling the governments of the United States, Japan and Europe. At the heart of the conflict is frustration in the United States at unsuccessful attempts both to curb the rising tide of Japanese microchips and to tighten its grip on its own components sold overseas.

The fears of the US semiconductor industry, which has accused the Japanese of 'dumping' microchips below their market value, had been partially allayed by an agreement signed with Japan last summer. But the Americans have not been able to satisfy themselves that the Japanese are adhering to the terms of the agreement — increasing the price of microchips while assisting US companies to penetrate the Japanese market — and at the same time have provoked the wrath of European allies who consider the pact a cartel.

The United States is now forced into trying other methods of protecting its home electronics industries and preventing military programmes from depending on imported products and technology because of a weakened infrastructure.

For its part, the European Economic Community (EEC) is making plans to take the US-Japanese microchip accord to a meeting of the General Agreement of Tariffs and Trade (GATT) next week to seek a ruling on the legality of the pact. It says the agreement is a breach of international trading procedures.

Meanwhile, a Defense Science Board Task Force report looking into dependence of foreign semiconductors for military hardware says that unless something is done quickly, the US semiconductor industry will die. The report urges the Pentagon to pump more than $1,000 million into a new institute for advanced, high-yield manufacturing techniques that will restore US self-sufficiency.

This concept differs from a similar industry-sponsored programme called Sematech because it focuses on generic process technology rather than large-scale production. The task force also encourages the Defense Department to spend $50 million per year to establish eight university centres of excellence in semiconductor science and engineering.

The report focuses on dynamic, random access memories (DRAMs). US manufacturers used to produce nearly all of the world's supply, but now they hold less than 5 per cent of the world market. Japan has taken over the lead in DRAMs, and is moving up in other areas of the industry.

Part of Japan's success comes from aggressive marketing and government support and protection for domestic industries. But the key, according to the task force report, is Japan's superior manufacturing practices. Japanese manufacturers have invested approximately 35 per cent of sales in plants and equipment, compared with 20 per cent for their US counterparts. Japanese firms also outspend US firms in research — 13 per cent compared with 10 per cent of sales. US research funds are spent on developing products for immediate sale; in Japan more is spent on long-term improvements.

To solve the problem, the report recommends that US industry be permitted to form a consortium called the Manufacturing Institute for Semiconductors. The Pentagon would provide a $250 million capital investment, and $200 million per year in contract support for five years. The consortium would not only assure US military needs, but would also help restore commercial industry. The report suggests that the institute's efforts be focused on advanced manufacturing techniques for the next generation of DRAMs, specifically the 64-megabit DRAM.

The Semiconductor Industry Association was quick to endorse the task force report. The association will decide on its plans for the future of Sematech at a board meeting in Washington next week. But for the present, the industry is still struggling.

The trade agreement with Japan worked out last summer has not brought hoped-for relief to the US industry. Association officials have accused Japanese manufacturers of violating agreements to stop dumping chips — selling them below their manufacturing costs. The industry has asked the government to impose financial penalties on Japanese manufacturers for these actions, and take steps to open Japanese markets for US chips.

On another front, Britain, like other European partners, has been questioning the right of the United States to demand that no 'American' technology be re-exported from Europe without the permission of the Department of Commerce. The issue came to the fore again last week with Mr Paul Channon, the UK Secretary of State for Trade and Industry, rejecting a US request for audits on British companies to monitor their behaviour.

Bill Johnstone & Joseph Palca

UK space plan for government go-ahead at last

London

THIS week, a cabinet meeting, expected to be chaired by Prime Minister Mrs Margaret Thatcher, will try to arrive at a decision on the future of Britain's space research and investment. A 15-year plan, drawn up by the British National Space Centre (BNSC), which has called for about £200 million a year, is to be discussed with ministers, with particular attention to be paid to the views of the ministries that might suffer if money were diverted to a space programme.

The Treasury is likely to be the most formidable opponent to the plan and is unhappy about any laxity in the Public Sector Borrowing Requirement (PSBR), despite disclosures last week that the government's finances are well in the black.

Thatcher could be asked to be the final arbitrator, as some government ministers are prepared to side with the Treasury if the alternative is reduced budgets. The space research and investment funds at present come largely from the Departments of Trade and Industry and Environment and the Ministry of Defence.

The BNSC plan requires not only the doubling of UK investment in space but also that it should control those diverse funds. That aspect of the plan is expected to be opposed by the respective departments and some of the research councils.

The BNSC had to postpone allocating industrial development contracts last month because of a lack of funds caused by the government's vacillations, and the centre was becoming increasingly embarrassed by its inability to commit Britain to the new projects to be undertaken by the European Space Agency (ESA), of which the United Kingdom is a member. BNSC officials have been meeting their European counterparts weekly since the beginning of the year, preparing for a full ministerial council meeting of ESA in June, but cannot outline Britain's space plans.

The government has been extremely reluctant to invest in research or industry if such funding can be acquired from the private sector. The BNSC, however, says that the government must take a lead in developing technologies and in research that shows no obvious commercial return.

The scientific advisers to the Cabinet Office prefer a more modest approach to Britain's space ambitions. The proposed BNSC budget is a commitment not favoured by the advisers. A plan in which spending would approach an annual target budget over a three-year period has more popular appeal.

Bill Johnstone

US call to end CFC emissions

Washington

Europe will this week be under strong pressure from the United States to take steps to control the discharge of chlorofluorocarbons (CFCs) into the atmosphere. The occasion will be a meeting in Vienna, starting 23 February, of the Vienna Convention on the Protection of the Ozone Layer, of which the European Economic Community (EEC) is a member.

The US delegation is calling for a freeze on the emission of CFCs, followed by a long-term planned reduction of emissions by as much as 95 per cent. This represents a considerable strengthening of the US position since the last round of negotiations in Geneva in December, when the participants got no further than deciding that something must be done.

Two bills introduced in the US Senate last week are designed to support the US negotiations. Both threaten to implement trade restrictions against countries that do not virtually cease production of ozone-depleting chemicals — brominated hydrocarbons as well as CFCs. Under the terms of the bills, US production will be decreased over a 6–8 year period to a ceiling of 5 per cent of present production. The chemicals are used in refrigerants, solvents and foam packaging for food; the use of CFCs in aerosol propellants was banned in the United States in 1978 and afterwards in Switzerland, Canada and several Scandinavian countries.

The sponsors of the Senate bills, Senators Max Baucus and John Chafee, cite a prediction of the Environmental Protection Agency that there would be 40 million excess skin cancers, resulting in 800,000 deaths in the United States alone over the next 80 years, if nothing is done to curb CFC emissions.

The situation is complicated by disagreements within the EEC about the seriousness of the problem. According to one Senate staff member, the United Kingdom and France are "not willing to admit there is a problem" of ozone depletion, while others did not attend the last round of negotiations. On the other hand, Denmark (which has no CFC industry to protect), has already banned the use of CFCs in aerosols — and may find other EEC nations taking action against it for restraint of trade.

The official US negotiating position in Vienna is less aggressive than the Senate bills. Ambassador Richard Benedick, leader of the US team, hinted last week that the United States would consider signing agreements with individual countries if no general agreement was reached.

Steven Dickman

Row takes new turn over US plagiarism of Soviet books

London

The case of Dr Hollis Chen, the Ohio University professor accused of plagarism (see *Nature* **324**, 198; 1986), has taken a new turn. Chen was accused, in the pages of the Moscow weekly *Literaturnaya Gazeta*, of having pirated two books by a member of the Byelorussian Academy of Sciences, incorporating them into his textbook *Theory of Electromagnetic Waves*. The attack was signed by the president of the Byelorussian Academy, Dr Mikolaj Barysevic, and a three-times State Prize winner, Dr Barys Sciapanau; Chen's defence was that he had never read the works in question, that he speaks no Russian, and that in any case he was writing a university textbook, not a scholarly monograph. He made no claim to originality, he said, and was not responsible for the dust-jacket blurb which suggested that this was the first time the subject was treated from a "coordinate-free" approach.

The second accusation, published in the *Literaturnaya Gazeta* on 28 January, and signed by two other Byelorussian scientists, raises a new charge. It attacks Chen's claim that he did not know the books, stating that a copy of one of the disputed works, the *Theory of Gyrotropy*, was mailed to him by the author, Dr Fiodar Fiodarau. Better known in the West under the Russian version of his name, Fedorov, Fiodarau maintains that in January 1978, Chen wrote to the *Navuka i Technika* press in Minsk to order a book entitled *The Theory of Optical Activity* of which Fiodarau was a co-author. This book, although advertised, had never been published and was superseded by the *Theory of Gyrotropy*. Accordingly, the publishers forwarded Chen's letter to Fiodarau, who replied by sending him a copy of the latter work, says Fiodarau. Chen admits ordering *The Theory of Optical Activity*, but denies ever receiving any acknowledgement of his letter, let alone a book.

Following the earlier article in *Nature* and a similar article in the *New York Times* by Dr Theodore Shabad of Columbia University, the University of Ohio appointed a special committee to look into the charges. The committee was composed of the head of the department of physics and astronomy, Dr Edward Sanford, and two visiting professors, Dr Clayborne D. Taylor of Mississippi State University, and Dr John M. Alexander of the University of Wales.

Their findings, in effect, deny the charges that Chen had copied four chapters almost completely, and that his diagrams and some 1,000 equations were copied unchanged. They conclude that "the test of plagiarism in the case of teaching material of the type found in textbooks, therefore, as distinct from the possible plagiarism of some completely innovative theory of technique, must be a subjective one, based on the degree and extent of the similarity involved". Chen's work, they said, uses a more modern mathematical treatment, and contains far more diagrams.

Nevertheless, the committee noted some "significant similarities", although not to the extent alleged in the Soviet charges. "Involved are two sections totalling about seven pages in length which are separated by completely disparate developments of the theories involved", they say, and while clearing Chen of the charges of extensive plagiarism, they allow that "there may possibly exist some grounds to believe that Chen made use of a small amount of introductory material from a secondary source" (which itself may have derived from Fiodarau).

The committee met before the appearance of the second *Literaturnaya Gazeta* article, and at that time, it seems, Chen made no mention of his 1978 letter to the Byelorussian publishers. The committee accordingly met again last Friday to discuss the second batch of charges, and again decided in Chen's favour.

So far, the committee has had no chance to see the *Theory of Gyrotropy*, which is not readily available in the West. One wonders, indeed, whether the cause of academic enquiry might not have been better served had the Byelorussian Academy made a discreet approach to Ohio University, submitting copies of the Fiodarau books and marking the disputed passages, rather than resorting to the Soviet press and then, via the Novosti Agency in London and the *Literaturnaya Gazeta* correspondent in New York, ensuring that western journalists noticed the allegations.

One would have preferred, too, a more academic and less polemical approach from the Soviet side — the second *Literaturnaya Gazeta* referred to this correspondent as "a certain" Vera Rich, cast doubts on her professional competence, hinted that *Nature*'s response was too rapid, and increased the number of allegedly pirated chapters from four to five. The University of Ohio, on the other hand, might have been well-advised, instead of an in-house enquiry, to refer the whole matter to some external body, perhaps to one of the many learned societies to which Chen belongs, and from which, the first *Literaturnaya Gazeta* article made clear, the Byelorussian Academy would like to see him expelled.

Vera Rich

International winners for Japan's version of Nobel Prize

Tokyo

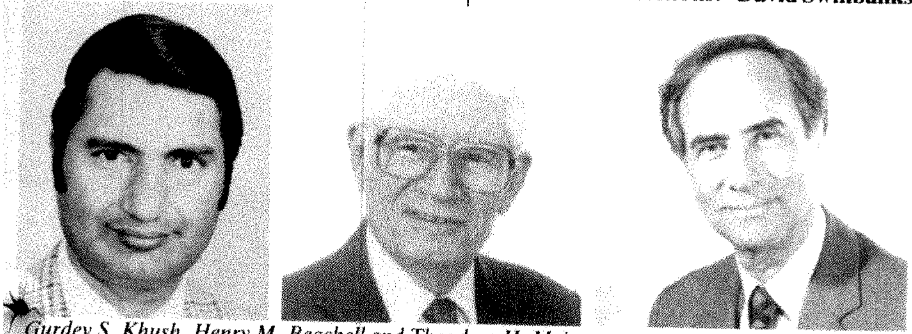
WINNERS of the Japan Prize, Japan's answer to the Nobel Prize, were announced last week. This year's prizes go to Dr Theodore H. Maiman, father of the laser, and to two noted agronomists, Drs Henry M. Beachell and Gurdev S. Khush. They will receive their awards on 14 April.

The prize, now in its third year, is worth Y50 million (US$330,000) and is awarded in two fields each year by the Science and Technology Foundation of Japan. The foundation was set up in 1983 with a donation of Y3,000 million from Matsushita Electric Industrial Co. But plummeting interest rates in Japan have eaten into the foundation's earnings and Konosuke Matsushita, founder of the giant electronics company and president of the foundation, recently supplemented the funds with a personal contribution of Y2,000 million.

The prize is awarded to "persons recognized as having served the cause of peace and prosperity for mankind through original and outstanding achievements in science and technology", and is oriented towards the applied sciences.

The two fields selected this year are improvements in biological functions and electro-optics. Maiman, a US citizen, won the prize in the latter category for his development of the ruby laser (*Nature* **187**, 493–494; 1960), and Beachell and Khush share a prize for their development of superior rice strains, which brought about a 'green revolution' in Asia.

Beachell, a native of Nebraska in the United States, developed the IR8 variety of rice in 1966 at the International Rice Research Institute in the Philippines. The IR8 strain has high photosynthetic efficiency and brought a rapid increase in rice production in tropical and sub-tropical environments. Khush, from India, succeeded Beachell in 1972 as head of the institute's plant breeding department and through crossbreeding of 13 parent plants from six countries developed the IR36 strain, which, as well as having high yield, excels in resistance to disease, insects and adverse soil conditions. **David Swinbanks**



Gurdev S. Khush, Henry M. Beachell and Theodore H. Mainman

Indian technology missions told to hurry up

Bangalore

MR Rajiv Gandhi, the Indian Prime Minister, is dissatisfied at the tardy progress of the technology missions meant to provide safe drinking water, better communications, universal immunization and vaccination by 1990 and increasing oil seed production. He has therefore called for a more urgent approach from his ministers and he has directed the ministries and departments involved in programme implementation to keep him regularly formed of progressed. At a review meeting of the missions held early this year, Gandhi made it plain that "ministers and bureaucrats are equally responsible for the successful implementation of these missions and I will not spare anybody".

He went on to say that the objectives of each mission must be quickly achieved. "The objectives have to be specific, tangible and measurable. It is imperative that specific time-bound tasks are spelled out clearly and allotted to specific institutions for implementation."

Eleven pilot projects are under way in the drinking water mission in 15,000 villages. The communications mission is chiefly aimed at increasing the efficiency of telegram delivery in 500 towns and cities and the target for the telephone network is to bring down the fault rate from 35 per 100 telephones to 10 by 1990.

The technology mission in vaccination and immunization plans to cover 92 million pregnant women and 82 million infants by 1990. The chief diseases to be covered under this programme are polio, measles, tuberculosis, diphtheria, tetanus and typhoid. In oil seeds, the mission is hoping for an output of 24–26 million tonnes by the year 2000, to which end work on development of high yielding varieties of groundnut, rapeseed, mustards, soyabean and sunflower has been intensified.

Radhakrishna Rao

Regulated misconduct

Washington

THE National Science Foundation (NSF) plans to adopt formal rules for the regulation of misconduct by recipients of its grants. The rules, formally approved by a meeting of the National Science Board last week and published in draft form in the *Federal Register* for 10 February, are essentially the same as those in force at the National Institutes of Health. Misconduct is taken to include fabrication, falsification and plagiarism or similar deviations, failure to comply with regulations for the ethical treatment of human subjects and animals, and the breaking of laws applicable to research. According to Erich Bloch, director of NSF, only two cases of misconduct of this kind have come to light among recipients of NSF grants. Under the new procedures, which are open for comment until 13 April, first responsibility for investigating allegations of misconduct will lie with the recipient institution, in the light of which NSF will decide whether to hold an investigation of its own. There is a formal mechanism for appeal. Anonymous informants will be dealt with confidentially. Penalties range from a reprimand to the cancellation of existing grants and the declaration that individuals or even institutions are ineligible for future grants. □

Canadian Advisory Board

Ottawa

CANADA's latest reorganization of research administration was signalled last week when the Prime Minister, Brian Mulroney, held the first meeting of the National Advisory Board on Science and Technology, which includes representatives of the Canadian scientific, business, labour and professional communities. The board, reporting directly to the prime minister, is intended as a "house of sober second thought" providing advice on science and technology policy, especially as these affect the international competitiveness of Canada's high-technology industries. Meanwhile, the draft of a comprehensive national science policy, drawn up after consultation with federal, provincial and territorial ministers, is being revised for publication in the early spring. The intention is that it should be the foundation of a "national policy action plan" to be worked out in the next two years. C.E.

New Woods Hole Director

Washington

THE trustees of the Marine Biological Laboratory at Woods Hole have elected Harlyn O. Halvorson of Brandeis University as the laboratory's new director. He will take up the post in August. Halvorson, who has been affiliated with Woods Hole since 1964, replaces J. Richard Whittaker, acting director since the retirement of Paul Weeks in October 1986. S.D.

Chiefs dominate indians at an annual science smorgasbord

Chicago

THE gamble by the American Association for the Advancement of Science (AAAS) to hold this year's annual meeting here in mid-winter appears to have succeeded; there has been no snow during the five-day meeting, while the biting wind off Lake Michigan has not been much worse than it can be in summer. The result is that there were roughly 3,400 members (a lot better than last year) at the smorgasbord of hard science made popular (or at least intelligible) and of public policy made technical. There were sessions on everything from particle physics and arms control to the state of Lake Michigan (whose level has risen 1.5 m in five years) and the chance to see night baseball at Wrigley Field, the home of the Chicago Cubs.

The most constant crowd was that at the neuroscience symposium organized by Dr Daniel L. Alkon of the Marine Biological Station at Woods Hole, a kind of current-awareness course equally suitable for outsiders and for those working in the field. And the highlight of that was the astonishing cine-film-footage from Thomas S. Reese (also from Woods Hole) of the movement of mitochondria and packets of neurotransmitter substances along the cytoskeletal microtubules with which the neuronal cytoplasm seems to be packed. It is not so much the measured speed (up to 40 cm a day) as the manner of travel that had Reese's audience spellbound. Mitochondria seem to wriggle their way along the microtubles like loose-fitting railway wagons along a track, sometimes half a dozen or more in line, occasionally changing track.

Other crowds were not so big (AAAS's worry) and seemed sometimes to be outnumbered by the speakers; the effect can be anticlimactic if a panel wishes to pose grand questions best suited to crowded halls, as did the speakers in the five days of talks about the US space programme after Challenger.

The government view is that the manned space station is the "cornerstone" of the next decade's programme for NASA (National Aeronautics and Space Administration), but academic researchers dissent. Ricardo Giacconi, developer of the Einstein X-ray satellite and now director of the NASA-supported Space Telescope Science Institute, flatly told a panel of government officials that there is "very little" support for the space station in the academic community. Giacconi's view is that NASA's commitment to people in space has caused the costs of scientific projects to skyrocket. While thrilling a larger audience with a vision of what trans-spectral astronomy beyond the atmosphere can accomplish, he complained that servicing the Hubble Space Telescope from the space shuttle will cost almost as much as building a second telescope.

Among the grand questions, that of how cold it will be after a nuclear war remains alive, even though S. Fred Singer, a physicist at George Mason University, Virginia, heaped criticism on models predicting severe cold for their failure to take account of the properties in the infrared of the smoke and ice-clouds that would form after a nuclear exchange. He argued that there would be a greenhouse effect, as a result of water vapour carried into the stratosphere coupled with smoke lower in the atmosphere, huge enough to turn nuclear winter into nuclear summer.

But Stephen Schneider, of the National Center for Atmospheric Research in Boulder, Colorado, accused Singer of raising an "infrared herring". Schneider says Singer's physical issues do not change predictions that temperatures would drop after a nuclear exchange. Schneider says a low smoke cloud's infrared properties would not be significant, although the ice issue may have some relevance. But, in any case, said Schneider, even a relatively small drop in temperature could be disastrous if it produced a frost during the growing season. Schneider believes a more serious issue yet to be given the attention it deserves is the effect of fog on post-war temperature.

Pre-war as well as post-war problems were examined if not solved at AAAS. On arms control, Roger Fisher, a professor at the Harvard Law School, said at one point during five days of discussion of security issues that US negotiators may be good at presenting positions, but are less good at plotting strategy for making progress. One way to succeed is to describe new positions without commitment. In the same vein at a different meeting, Professor Thomas Schnelling, also of Harvard, had asked for a "moratorium on arms control negotiations" not only because of the damage they can do but because they divert attention from other beneficial courses of action.

Of necessity, there were not many jokes in these sessions, and even Academician R.Z. Sagdeev's joke was a black one. Seeking a simple model of the doctrine of mutually assured destruction, he told of the Russian couple who, when asked at their silver wedding how their marriage could have survived all the tribulations of married life, replied that they had filled their basement with dynamite, and that each had had a button.

John Maddox & Joseph Palca

Czechs agree with Poles over water pollution

London

THE Czechoslovak Minister for Forestry and Water Management, Franktisek Kalina, last week officially "deplored and apologized for" the effects of the oil spillage at the Kunice cement works last November, which polluted the Oder and caused considerable damage in the Polish province of Katowice. After a two-day meeting in Krakow with the Polish Environment Minister, Stefan Jarzebski, Kalina said that steps had been taken to prevent a similar spill in the future, that both sides had agreed to the expansion of joint monitoring of border and trans-boundary rivers and that the obligation to pay for cleaning up pollution would fall on the country from which the pollution had come.

Pollution of Czechoslovak origin is a long-standing grievance to the Poles. In the mid-1970s, when Polish censorship imposed strict controls on discussion in the media of industrial degradation of the environment, the ban did not apply to pollution originating from Czechoslovakia. In the case of the recent spill, there were two main causes of tension. One was disagreement between the two sides about the extent of the spill; the other was that the Czechoslovak authorities had failed to provide the Poles with adequate and timely warning of the spill.

Although, on 9 December, the Polish government press spokesman, Mr Jerzy Urban, dismissed western reports that the spill had caused a major "conflict" between the two socialist states, a few days later the Warsaw correspondent of Czechoslovak television, Ratislav Baja, noted Polish complaints that the Czechoslovak authorities had provided only "incomplete" and "distorted" data. Similarly, on 19 December, the Polish Deputy Premier, Jozef Koziol, answering questions from the Parliamentary Commission on Environmental Protection, described the information provided by the Czechs as "strongly understated".

The Czechoslovak answer was that the Poles had measured the spill wrongly, as what they had collected was not oil but an oil-water mix — and that some of the pollution was probably of Polish origin anyway. There has been no attempt by the Czechs to explain the delays in information and, ironically, on the eve of the Krakow meeting, the Warsaw daily *Zycie Warszawy* reported yet another Czech spill near the city of Ostrava, which although not large was once again disquieting, as the Polish environmental services first learned about such events "from chance observers".

Vera Rich

Towards truth in advertising in US university education

Washington

WILLIAM J. Bennett, Secretary of Education in the Reagan Administration for the past two years, is a name with which to inflame academics. Bennett's pitch is that the quality of higher education in the United States is deficient because academics neglect their primary responsibility (to teach) and that universities are bilking the US economy by increasing tuition fees unjustifiably. But he says that the *New York Times*, not he, was responsible for the word "greed" in the headline above a statement of his case last week.

Contrary to academic suspicion, Bennett does not have horns growing out of his head. His special unpopularity on the campuses of the United States may stem from his own academic background — Williams College, a PhD in philosophy at Austin, Texas, a law diploma at Harvard Law School and some years of teaching and administration at Boston University, President John Silber's centre of successfully self-proclaimed excellence; apostates are rarely loved. In 1981, Bennett was President Reagan's first appointment as chairman of the National Endowment for the Humanities.

Bennett makes no bones about his objectives: "I'm deliberately setting out to improve the quality of higher education", but quality is carefully defined. Bennett says the essence of his complaint is that universities are failing to deliver what they promise. He says there is a huge gap between the rhetoric of university catalogues ("we collect them here") and the actual experiences of undergraduates. He cites as evidence the Boyers report from the Carnegie Commission for Higher Education last November, with its account of the incidence of student dropouts and of discontent among those who stay.

If colleges accurately advertised their wares, one gathers, the secretary would be content, knowing that institutions offering second-quality goods would then not find students. In the marketplace for higher education, Bennett is the campaigner for better advertising standards. "To thine own claims be true" is his motto.

But are not many institutions providing an excellent education? Bennett says his complaints apply to "most places", not to all. Faced with the example of one university given a glowing report card by an external invigilating group, Bennett quickly says "I'll take some credit for that". After two years of demanding systematic evaluation of universities, "I think we've won that argument".

On research, Bennett deplores the ease with which universities can upgrade their status, duplicating each other's efforts and increasing the demands on federal funds for research grants. "North Carolina used to have two universities, now it has seventeen", he says, offering as an illustration of the research contagion the way in which state system colleges give themselves airs above what used to be their station.

Bennett is offended by the "higher education lobby, which has done a very good job", and almost sympathetic to the US Congress, "which has to face it year after year". "If you want to use the word greedy", the lobbying on behalf of universities during the debate over last year's Tax Reform Act is where it applies, he says.

In the event, the private universities represented by the American Council on Education appear to have lost most of their claims for tax exemption for charitable gifts and the capital appreciation of property, while not all scholarships and fellowships will in future be tax-exempt.

In reality, Bennett says, it is "balderdash" for the universities to act poormouth when higher education has an aggregate income of more than $100,000 million a year. His constant theme is that there would be plenty of money for the universities if only it were wisely spent.

Bennett's draft budget for the financial year beginning next October would make the federal government, which even now contributes only 6–7.5 per cent of the total, an even smaller player. The administration hopes it will be allowed to save $5,500 million from educational programmes, but the Secretary of Education seems less than confident that Congress will allow him to spend so little.

There will be a bitter argument over financial support for university students. Bennett makes no bones of his objectives; access for young people likely to benefit from higher education, but not at the expense of "taxpayers who never go to university". He declares that there cannot be much wrong with a system in which 60 per cent of young people at least begin on higher education courses of some kind, but insists that, in equity, the burden of student support should be shifted from the present system of modest grants and interest-subsidized loans to one in which interest would not be subsidized but the rate of loan repayment determined by a graduate's salary.

William J. Bennett — inconoclast or populist?

Bennett will not be discomfited by the budget battle ahead. Down-to-earth though he may be, there is a touch of the messianic about him, as if he has a vision of how the universities of the United States could prosper in a free market for higher education. If unsurprised that most academics do not share his vision, he talks as if it is only a matter of time before they are converted. He has one powerful influence on his side — the knowledge that in the climate of disappointment that marks the beginning of the next presidential election campaign, the charge that the universities of the United States have shortchanged their clients may strike a popular chord. But Bennett has a soft spot for the problems of research in the humanities, including Greek and Latin, which may yet win him unexpected friends. **John Maddox**

New Indo – Soviet space agreement

Bangalore

As part of an agreement between the Soviet Union and India to improve telecommunications between the two countries, the Soviet posts and telecommunications ministry will give technical help in setting up an Intersputnik Earth station in India. India is also likely to lease transponders on a Soviet communications satellite, which will make possible an exchange of television and radio broadcasts.

During the visit of the Soviet leader, Mr Mikhail Gorbachev, to India at the end of last year, the Soviet delegation suggested the establishment of a space centre in India to train cosmonauts from developing countries.

India has also concluded an agreement with the Soviet agency Glavcosmos for the launching this year of India's three-axis-stabilized operational Earth observation spacecraft, IRS, from the Soviet Union. India is said to be the first ever customer to hire the launch service offered by Glavcosmos and this will be the first commercial launch of an Indian spacecraft from the Soviet Union. India's first satellite, Aryabhata, and the experimental Earth observation spacecraft in the Bhaskara series were put into space free of charge by the Soviet Intercosmos vehicle.

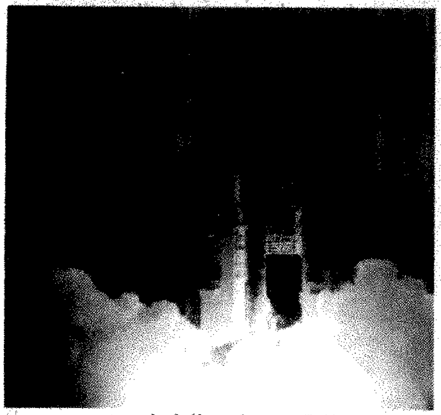
Radhakrishna Rao

Japan's successful launch of first marine observation satellite

Tokyo

JAPAN's first marine observation satellite, MOS-1, was successfully launched from the National Space Development Agency's (NASDA) space centre in Tanegashima island off Kyushu on 19 February. The launch marks the end of an era of total dependence on US rocket technology and gives another boost to the morale of Japan's space engineers.

The satellite, named Momo (peach blossom), lifted off at 10.23 a.m. local time aboard a two-stage N-II rocket. After passing over Australia and Antarctica, the 740-kg satellite separated from the second stage over South America and entered a Sun-synchronous polar orbit at an altitude of just over 900 km about an hour after launch. Signals from the satellite were picked up immediately after separation at Kourou station of the European Space Agency in French Guiana.



Momo is equipped with three scanners: a multi-spectral electronic self-scanning radiometer with 50-m resolution in the visible and near-infrared to monitor water turbidity, red tides, waves and ice distribution over the sea, and vegetation, geology, water resources and snow cover over land; a visible and thermal infrared radiometer to scan snow, cloud, ice and water temperature distribution; and a microwave radiometer that will make cloud water content, rainfall and snowfall measurements. The scanners lack the resolution of France's SPOT-1 satellite, which can pick up objects 10 m across when in panachromatic mode. Momo is the world's only marine observation satellite following the demise of the US's Seasat-1 shortly after launch in 1978. And its Sun-synchronous polar orbit allows coverage of almost the entire surface of the globe, bringing the satellite back to the same spot every 17 days. In addition to NASDA's Earth Observation Center north of Tokyo, ground stations in Spain, Norway, Thailand and the Antarctic will receive data directly from the satellite.

The N-II rocket that carried the satellite aloft is based entirely on US rocket technology including the inertial guidance system which is a 'black box' taken from the US Delta rocket. But this is the last N-II launch and in future NASDA will use rockets largely or entirely developed in Japan. The H-I rocket, which was successfully launched last summer, has only US components in the first stage; the H-II, due to be completed in 1992, will be entirely made in Japan.

NASDA executive vice-president S. Sonoyama is noncommittal on whether Japan will enter the commercial space race with the H-II. But other officials know they must reduce launch costs for geostationary satellites from the present level of Y30 million ($200,000) per kg for the H-I to Y8 million per kg to be commercially viable.

The H-II will have a payload capacity of only 2,000 kg for geostationary satellites, half the capacity of Ariane-5. But Sonoyama believes conventional geostationary satellites will not rise above 2,000 kg because of difficulties of launching heavy satellites; instead he sees the future in multipurpose space platforms.

The Ministry of International Trade and Industry, the Institute of Space and Astronautical Sciences affiliated with the Ministry of Education, Science and Culture, and NASDA are jointly developing an experimental space platform that will be launched by 1993 at the earliest by an H-II rocket and brought back by the US space shuttle. Although current specifications for the H-II limit it to satellites up to 3.7 m in diameter, plans are being made to increase this to 4.6 m for space shuttle compatibility. And the H-II could launch large payloads (up to 4,000 kg) by augmenting the second-stage engine and adding boosters. Thus the next generation of satellites does seem within Japan's future launch capabilities.

The only remaining barrier to Japan's entry to the commercial market is the fishermen of Tanegashima, who restrict NASDA to two 45-day launch seasons in summer and winter, claiming that falling rocket boosters damage their fishing grounds. But even this may not be a problem. Masashi Motchizuki, director of the H-II launch vehicle group, confidently predicts that 10 launches could be made during the summer season alone.

Since Japan launched its first satellite in 1970, there has been only one failure out of 35 satellites, an experimental communications satellite launched in 1979. In this age of space disasters and soaring insurance costs for satellites, reliability will probably be Japan's strongest selling point.

David Swinbanks

Safety fears in nuclear debate

London

THE safety risks of nuclear energy have still not been properly assessed and there is no proper emergency procedure to cope adequately with another Chernobyl. These fears surfaced in public debate last week as British parliamentarians from all political parties expressed the growing disquiet among some members of the public about the safety of nuclear power.

At the beginning of the week, the House of Commons debated the findings of the 109-chapter report, prepared by Sir Frank Layfield, on the proposed pressurized-water reactor (PWR) to be built by the Central Electricity Generating Board (CEGB) on the Suffolk coast. Despite the opposition, there is little doubt that the government will approve the reactor, estimated to cost about £1,300 million.

The leader of the Liberal Party, Mr David Steel, was typical of those who consider the conclusions of the Layfield report on safety to be inadequate. In a letter to Mr Peter Walker, the Energy Secretary, Steel demanded that the risks of civil nuclear power be assessed and made public: "It [the report] appears to suggest that Sizewell B will be acceptably safe only if the economic benefits are large enough to justify the scale of the risk involved. Or, put even more directly, that there are safety risks involved but that these will be acceptable if the economic benefits are big enough."

The public debate on Sizewell B resurrected the fears about Chernobyl. Although the National Radiological Protection Board (NRPB) tried to inform all the local authorities seeking guidance in the immediate wake of the disaster, the "arrangements" to cater for such an emergency proved inadequate.

As a consequence, NRPB has advised the Department of the Environment, which is drawing up the new procedures, to create a national emergency telecommunications network.

Bill Johnstone

India establishes countrywide satellite computerized database

New Delhi

A NATIONWIDE satellite-based computerized information network is being set up by the Indian government to provide its planners with instant access to information needed for decision-making and monitoring of development programmes.

By the end of this year, government offices in each of the 430 districts, the 22 state capitals and major cities will be hooked into a vast information network that will move data from virtually any part of the country to the offices of ministers and their secretaries in central and state governments.

The man behind the $70 million project is Dr N. Seshagiri, director of the National Information Centre (NIC) created by Mrs Indira Gandhi in 1977. NIC now services 62 central government departments in New Delhi through a cable and VHF network linking computer terminals in individual departments with NIC's 170/720 computer, the biggest in India, which stores some 200 databases covering crucial sectors of the economy.

NIC's computerized management information has already proved valuable. For example, the performance of power plants is monitored daily, trade figures are updated every day and performance indices of all public-sector undertakings are regularly tracked. The 1987 budget, to be presented this month by the Prime Minister, Mr Rajiv Gandhi, will also be based on data drawn from NIC's computer. According to Seshagiri, NIC's services are now being extended to cover the whole of India with the help of the satellite.

The new project will ensure the creation of district databases on health, agriculture, rural development, employment and other relevant social and economic matters; they will be stored in locally made microcomputers in the offices of district collectors. Four large mainframe computers (NEC-1000) imported from Japan have already been installed in Delhi, Pune, Bhubaneswar and Hyderabad to store databases from the regions.

Within the next two months, superminicomputers will be installed in the offices of the chief secretaries in all the state capitals. These computers are being made by the state-owned Electronic Corporation of India under licence from the Control Data Corporation of the United States.

Except for the imported master Earth Station at New Delhi, all other computers in the network will be linked to the satellite by micro Earth stations and two-way roof-top antennas. The first batch of 120 micro Earth station antenna systems are being imported from the Equatorial Corporation in the United States. The rest will be supplied by Indian Telephone Industries in Bangalore, which has purchased the technology from the US company.

According to Seshagiri, the network will become operational in May, using a leased Intelsat transponder over the Indian Ocean. But NIC hopes to get a loan from the World Bank to acquire and launch its own satellite, Nicsat, in collaboration with the Indian Space Department, by 1990. Nicsat will operate in the Ka band (20 GHz), making possible the use of still smaller (50-cm diameter) dish antennas costing less than $2,000 each.

Seshagiri believes India is the first country to establish a satellite-based information network dedicated to government decision-making and to toning up the sagging administrative machinery. "Some 10,000 government-aided projects are being implemented in various districts, but central ministers have no fast means of monitoring the time and cost overruns", says Seshagiri. He says the cost of the network will be recovered several times over by better management of multimillion dollar development projects that now languish because of delays in decision-making.

States that were initially hesitant to share information with the centre have joined the network after an assurance from NIC that the autonomy of the federal structure will be respected. No data relating to police or security matters or that violates the privacy of individuals or organizations will be collected. Seshagiri says 85 per cent of information in databases will be open to officials; the remaining 15 per cent will be secure and available only to specially authorized personnel. About 1,300 government officers have been trained in database creation and NIC hopes to train several hundred more soon.

At the same time, the state-owned Computer Maintenance Corporation of India is setting up a network called Indonet for public use. It provides computer booths throughout the country that can be used by, among others, small businesses unable to afford their own computers. The fourth-generation IBM computers at Bombay, Hyderabad, Delhi, Calcutta and Madras that form Indonet will eventually be linked via satellite and expanded to cover 35 cities.

K.S. Jayaraman

Schools wooed for science teaching

London

THE promotion of science in British schools was given a boost last week with the launch of two programmes to attract unconfident teachers to science and to encourage them to include it in their curriculum.

The first is a comprehensive basic science teaching kit in the form of teachers' notes and colourful learning cards for children. The kit, prepared by the British Gas Corporation, and designed for young children, will outline the basic concepts of air, flight, forces, heat, water and 'ourselves'.

Another project is designed to promote education in science using information stored on a computer database. The project, which will be funded by the Department of Trade and Industry until April next year, allows teachers with microcomputers and modems to 'dial' into the system to obtain teaching aids.

The system, called the National Educational Resources Information Service, will have about 500,000 'resources' listed on the database by 1988. The data will be housed in the computer of the Open University.

Bill Johnstone

Teacher union issues AIDS guidelines

London

THE National Union of Teachers (NUT), the negotiating body for most of Britain's schoolteachers, has published guidelines on AIDS (acquired immune deficiency syndrome) to "reassure members and dispel myths about the spread of the disease".

According to the NUT deputy general secretary Doug McAvoy: "It is vital that teachers know the facts. The NUT's notes on AIDS should reassure members that they need not worry about contracting AIDS in their contact with pupils." Teachers should be entrusted with confidential information on children's health, and confidentiality must be maintained to protect children from victimization as a result of unfounded fears of infection.

The guidance notes, to be distributed to the NUT's 650 local associations and branches, focus on homosexual and bisexual men, drug addicts who share needles, and haemophiliacs as the 'risk groups'.

Evidence shows, says the union, that children who carry human immunodeficiency virus pose no risk to other children or adults in a normal school environment and should attend school normally.

The union says: "None of the identified cases of HIV infection in this country or the United States is known to have been transmitted in a school setting, nor has the virus been spread through casual personal contact. There is no reason for teachers to be concerned about handling objects used by an infected person and no need for separate toilets or crockery and cutlery".

Bill Johnstone

Antimatter underestimated

Sir—The first published account of success in immobilizing antiprotons in an electromagnetic trap has been the subject of a recent leading article in News and Views (*Nature* **324**, 299; 1986). Although this article gives a good overview of the scientific meaning of this historical achievement, it gives in our view a rather misleading perspective on its likely military significance.

It is true that for some applications of antimatter, such as antiballistic missile or spacecraft propulsion, relatively large amounts of antiprotons are required. For most other cases, the useful amounts are usually much smaller. For example, we have calculated that less than a microgram of antiprotons is sufficient to trigger a thermonuclear explosion or pump a powerful X-ray laser[1,2].

But antimatter is not only the most portable of all high explosives, it is also the only feasible portable source of muons. In every antiproton annihilation, on average three muons are produced. These could be used to induce muon-catalysed fusion reactions in a deuterium–tritium mixture, an attractive solution for a low-weight space nuclear reactor that could be operated in a continuous or pulsed mode.

Furthermore, by collecting and cooling the muons (an easy task compared with that of cooling antiprotons) a very intense beam could be formed and sent into the atmosphere to guide, over a range of more than 10 km, a series of powerful electron or proton beam pulses towards a target. More simply, stopping the muons in a suitable material would generate an extraordinarily effective X-ray lasing medium, for the two-microsecond lifetime of the muonic atoms.

In outer space, a very low intensity burst of antiprotons would be most suitable for active warhead/decoy discrimination. In this and the two previous examples, the amount of antiprotons needed is of the order of *nanograms* per engagement. Conservative estimates of the technical problems involved in producing and manipulating *microgram* amounts of antimatter per day show that known technology is only a couple of orders of magnitudes away from meeting the challenge[2–4].

We are very much concerned by the implications for nuclear weapons proliferation of the indisputable scientific feasibility of several antimatter weapon concepts. To develop such weapons would add considerable impetus to the current arms race. We call for an immediate ban on all antimatter-related research, especially as this work is fundamental to many third-generation nuclear weapon systems.

André Gsponer
Jean-Pierre Hurni
Independent Scientific Research Institute,
15, Charles-Galland,
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SDI boycott

Sir—Your leading article (*Nature* **323**, 746; 1986) questioning the wisdom of a boycott of the Strategic Defense Initiative (SDI) misses the point. Scientists who oppose SDI funding do not accept the premise that knowledge is worth gaining for its own sake, irrespective of the ultimate social consequences. We can wait to obtain some knowledge and its gains.

Of course academic researchers should decide for themselves what research they should undertake, but they should also strive to ensure that the knowledge they gain is not applied to ends that are not in the interests of humanity, questions of nationalism aside. Refusing SDI funding is one way of expressing opposition to the political control of science.

We in New Zealand admire the refusnik mentality and although there is no prospect of our being offered SDI money, scientists have played an important role in bolstering the government's policy of refusing port visits by nuclear weapon-capable vessels, of the Royal Navy and the United States Navy in particular.

Peter Wills
Department of Physics,
University of Auckland,
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Auckland,
New Zealand

In the greenhouse

Sir—T.R. Vidyasagar (*Nature* **323**, 390; 1986) suggests a switch to a vegetarian diet and reforestation of the 40 per cent of present agricultural land so freed as a way to decrease atmospheric CO_2. This short-term measure could be supplemented by fertilizing the world's forest and pasture soils, where applicable, with nutrients from city drains, combined with pelleted spores and seeds of soil-enriching plants. Only a light dressing of suitably prepared and selected material would be required to fortify their nutrient cycles and make them more productive for many years. Many parts of the oceans would respond to similar treatment by producing more fish, seaweed, corals and carbonaceous sediments, the latter being the more permanent sink for CO_2.

Perhaps floating lagoons made to transport sewerage could be towed like icebergs (used for soft water irrigation), to chosen sites, where the surface waters would be fed by trickle and slow release methods. Natural upwelling of nutrient-rich bottom waters of the ocean greatly increase their production of living organisms, (J.H. Ryther *Science* **166**, 72–76; 1969) and artificial methods could be developed to extend this process.

I look forward to a time when it is possible by careful balancing of the nutrition of *p*H of the biosphere in these and other ways to treble its content of unoxidized C, as has been accomplished on farms and gardens around the world in the course of a few decades.

R. Parr
Arrington Bridge,
Arrington, Royston,
Hertfordshire SG8 0AE, UK

First among equals

Sir—in a recent leading article (*Nature* **324**, 509; 1986), you ask "Should editors now seek to protect authors from themselves by banning the use of the word 'first'?". My reply is: would you care to join the *Proceedings*, which has had such a policy for some years?

I do not know exactly when the *Proceedings* first published priority statements. There is a 1977 memorandum in the files from Bernard K. Forscher, then managing editor, to Robert L. Sinsheimer, then chairman of the editorial board, suggesting such a policy (which may simply be the earliest available written expression of a previously unwritten policy) and a letter from Sinsheimer concurring. Since 1983 at least, the Information for Contributors has stated "The *Proceedings* does not print priority statements". Thus, one of the duties of our editorial staff is to remove such statements. Most authors accept this change with equanimity; for those who do not, we permit the qualified statement "So far as we know, this is the first...".

A policy that is much more difficult to implement is our proscription of statements of novelty. I admit that this has been unevenly enforced, although in the past year we have become more strict. Novel fragments, novel genes, novel hypothesis — the term is usually a cliché occasioned by the author's desire for an adjective, as our stated policy is to publish reports of exceptional importance and novelty. Again, would you care to join us?

Frances R. Zwanzig
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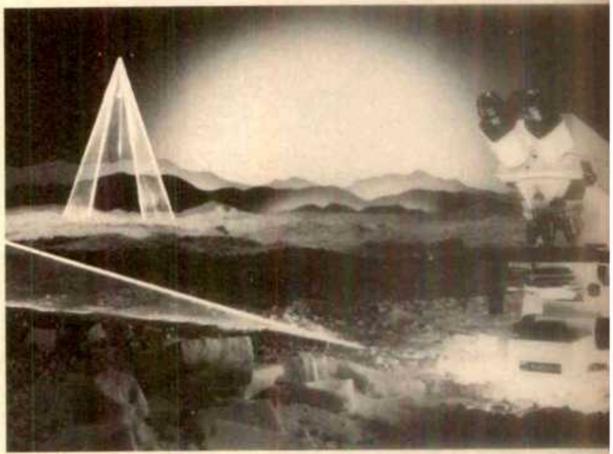

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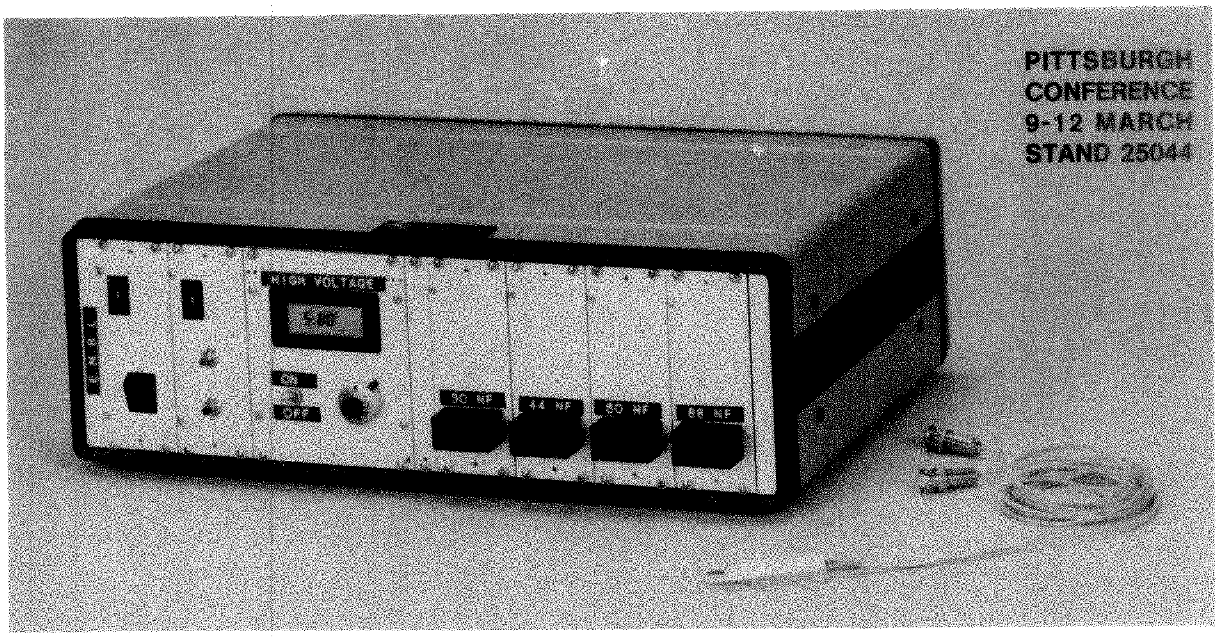

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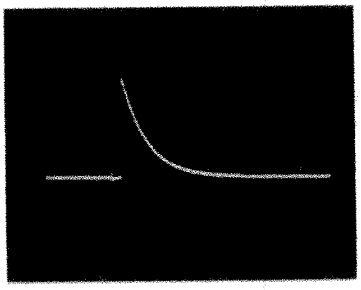

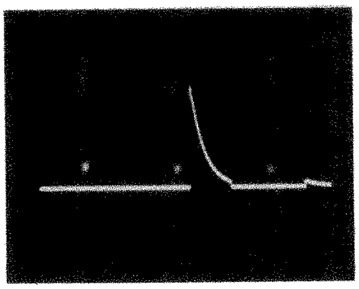

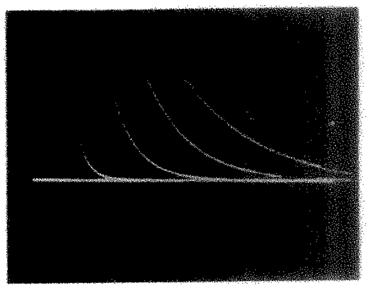

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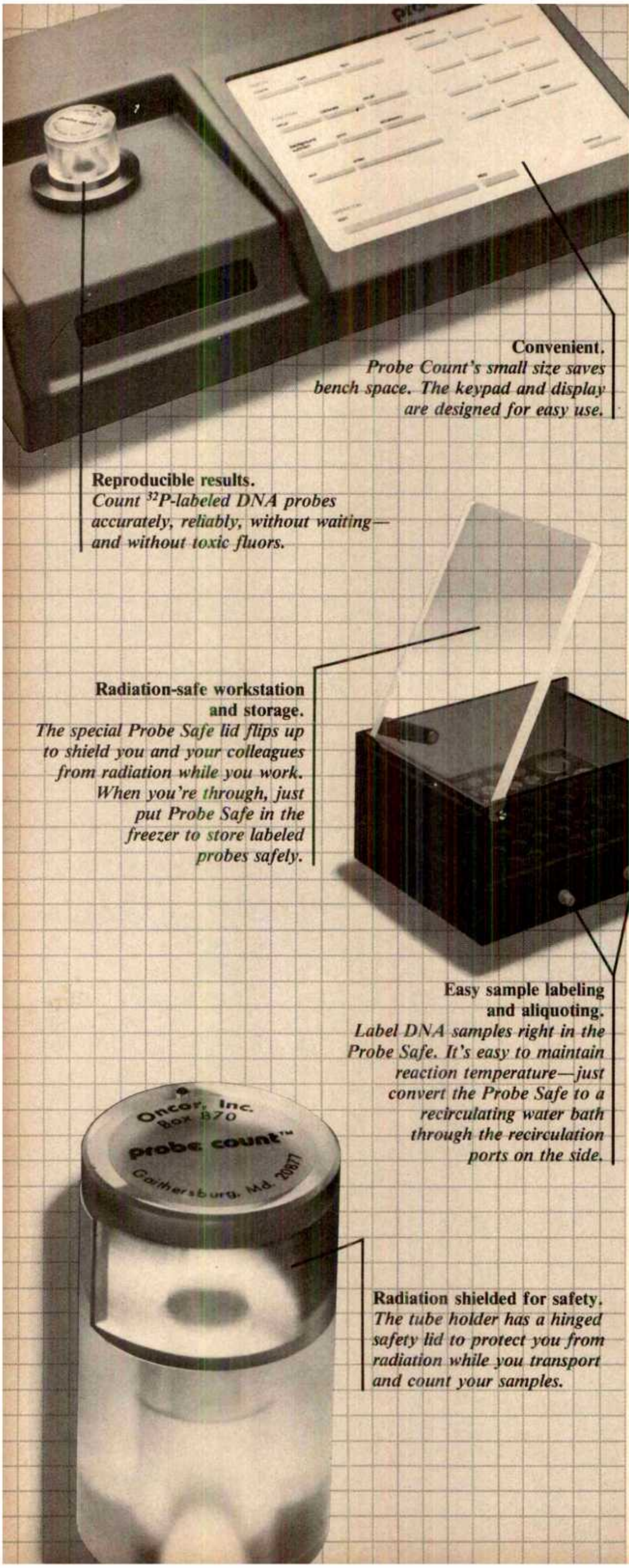

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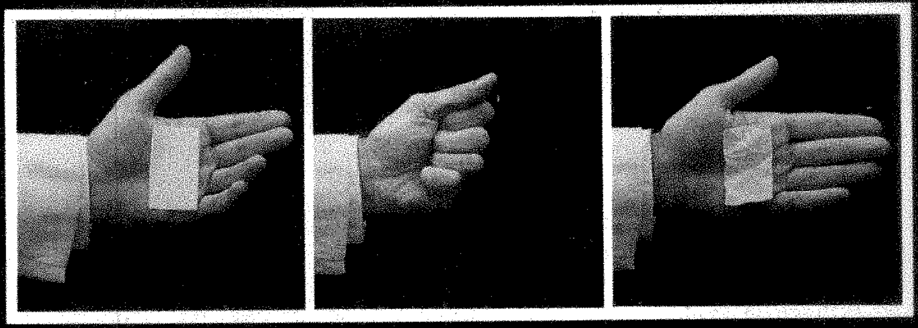

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Spring Harbor Laboratory chose a topic that most certainly will be returned to over and over during the second
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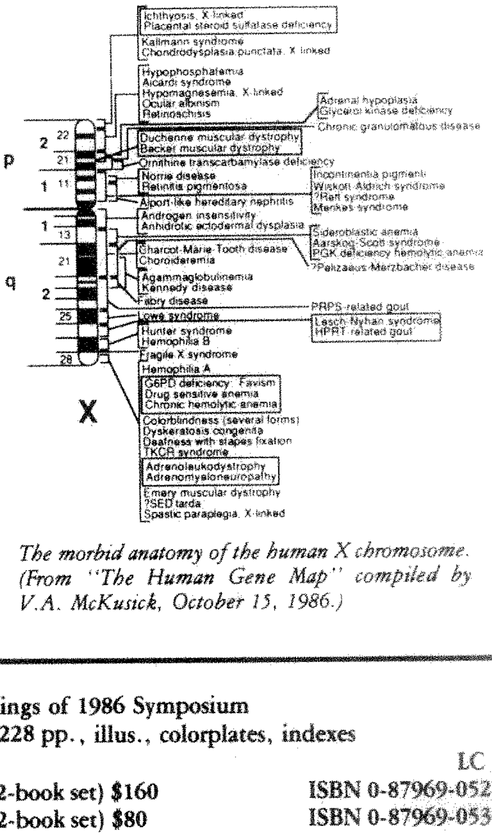

The morbid anatomy of the human X chromosome. (From "The Human Gene Map" compiled by V.A. McKusick, October 15, 1986.)

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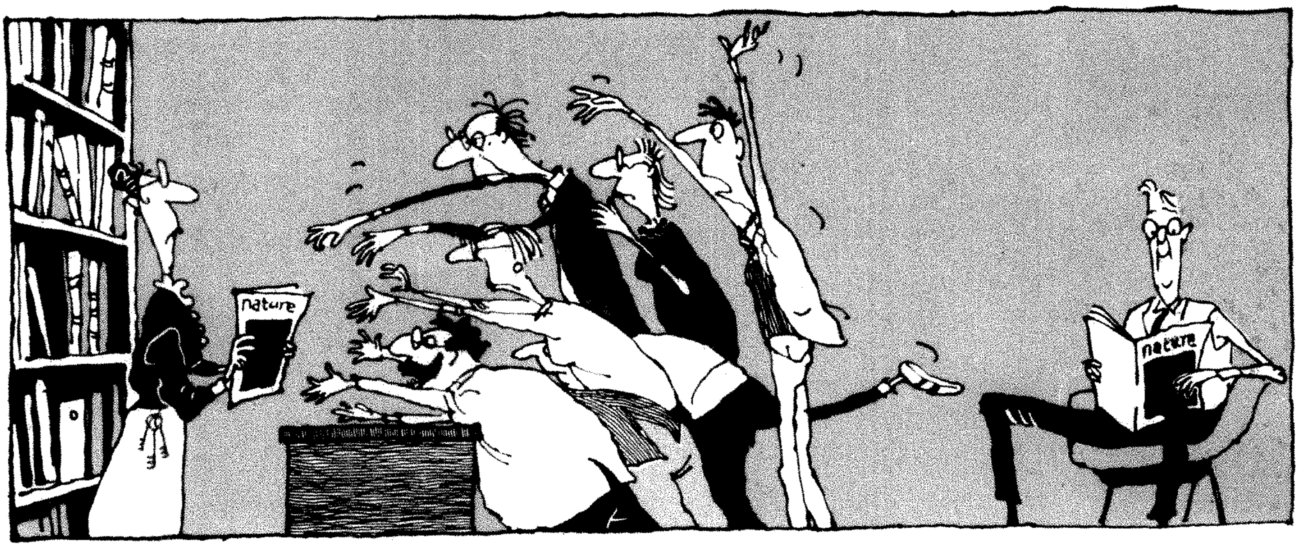

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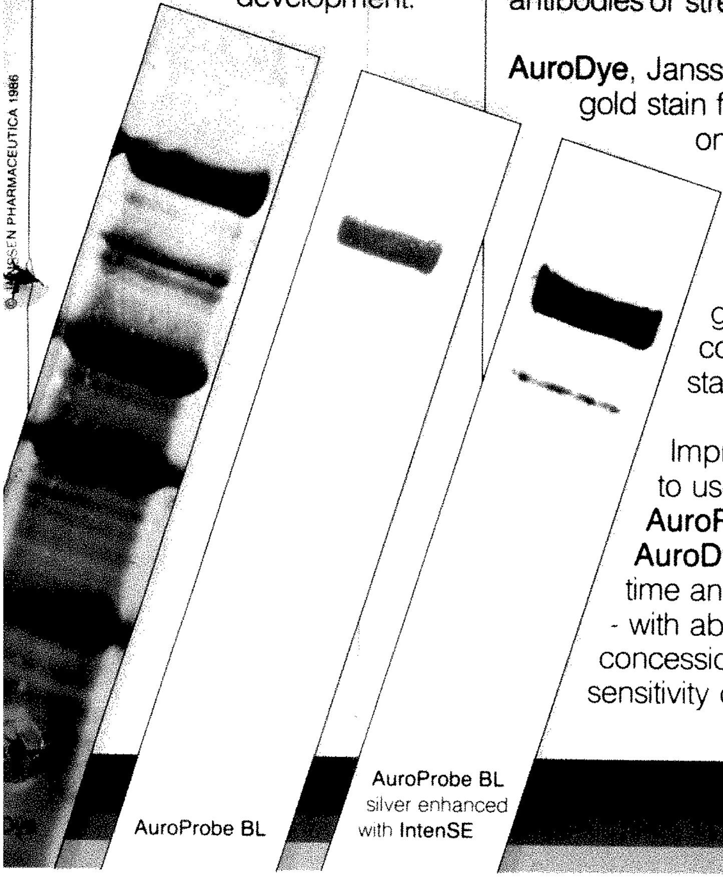

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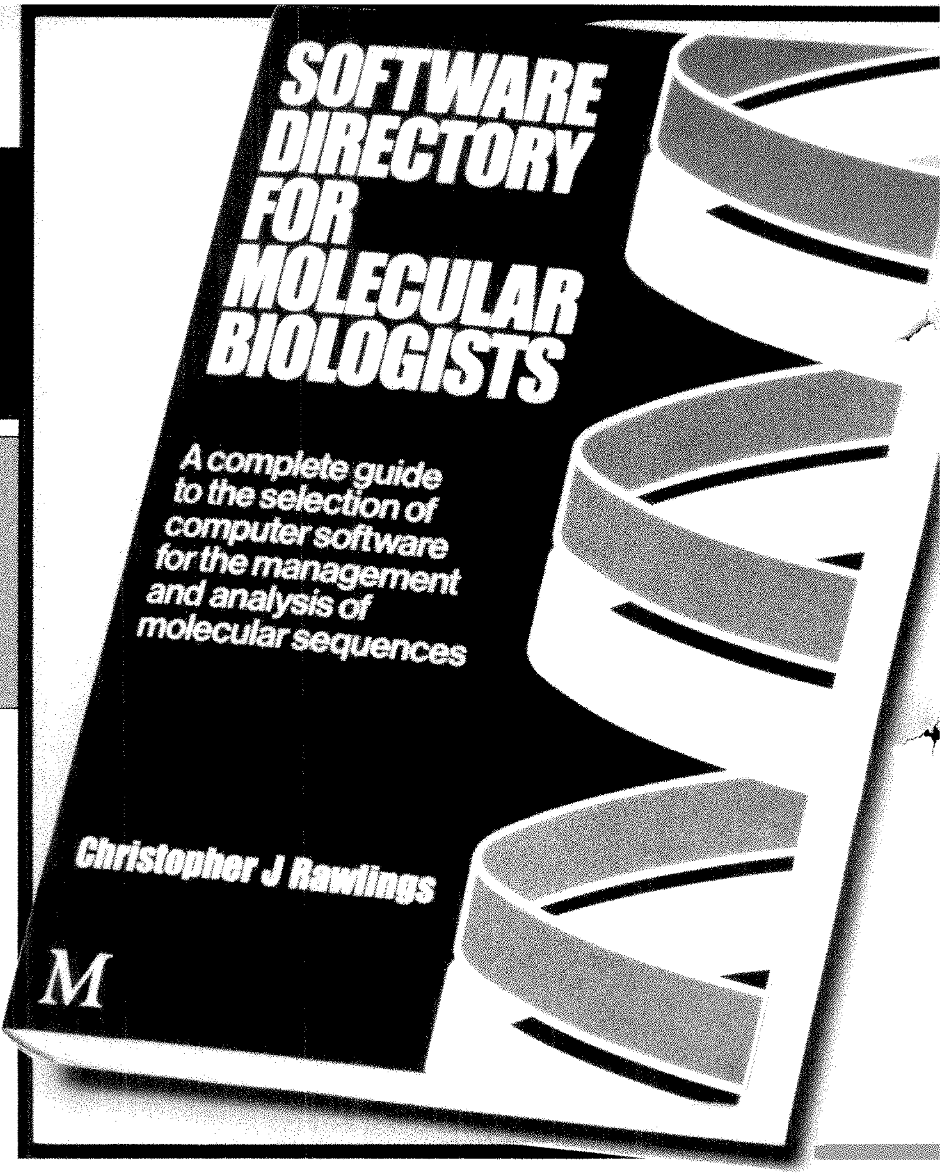
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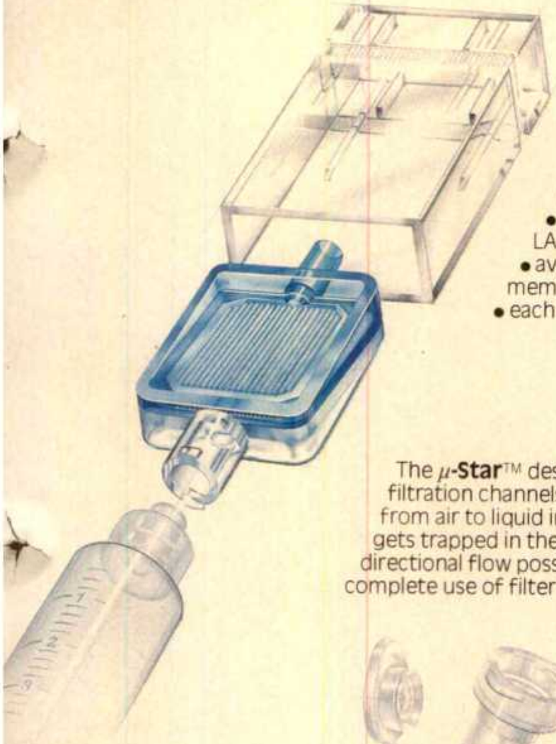

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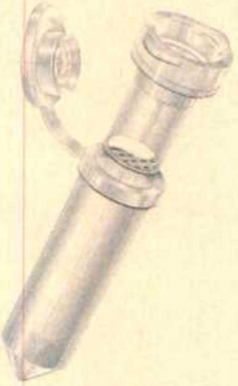

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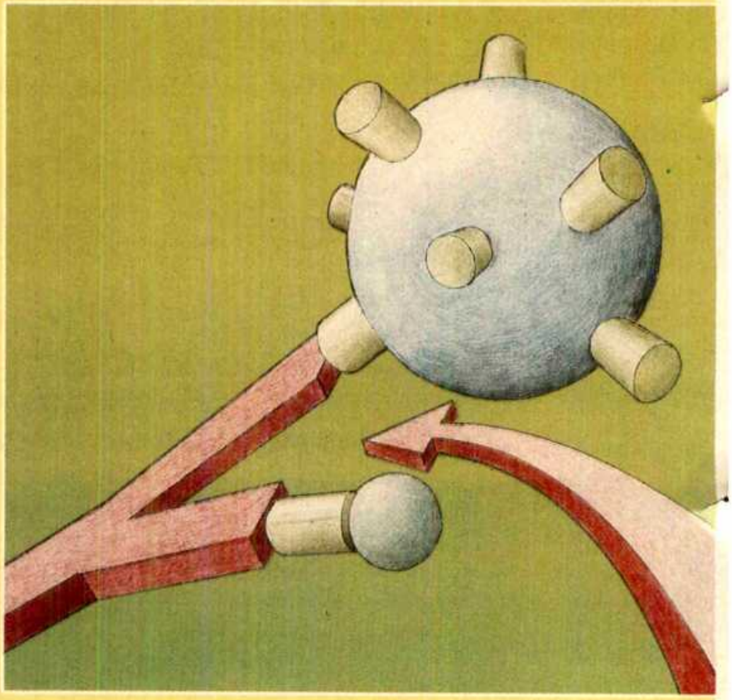

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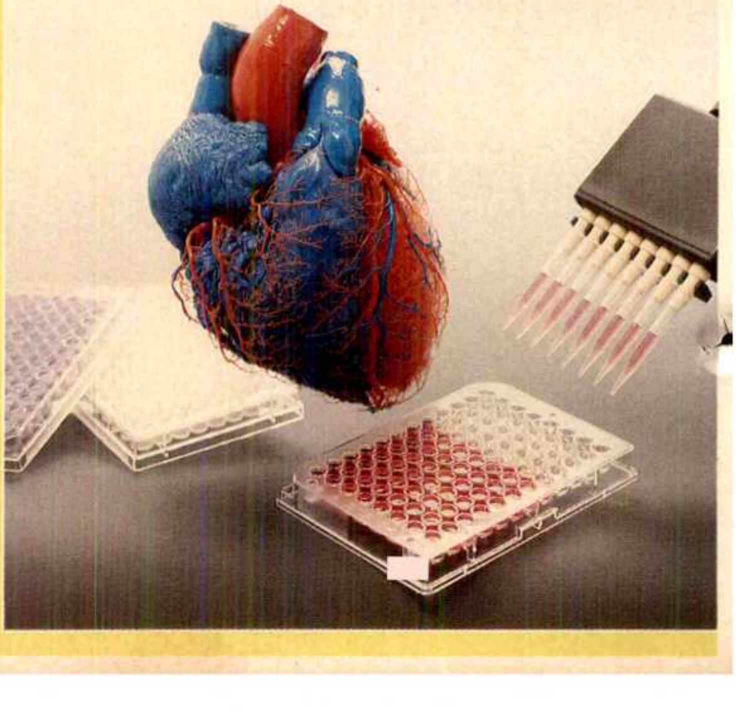


Reader Service No.42

Molecular genetics of the mind

The use of DNA markers has shown that manic-depressive illness can be caused by a single gene. These markers will not be useful for screening but may lead to fundamental insight into the disease.

THERE has for some time been good evidence that individuals in some families may be genetically predisposed to a spectrum of mental diseases of which the most distinctive is manic-depressive psychosis. About two years ago, Daniela Gerhard and colleagues published an abstract suggesting that manic-depressive disease among the Old Order Amish of Pennsylvania might be traceable to a single gene on the short arm of chromosome 11 (Gerhard *et al. Am. J. hum. Genet.* **36**, 35; 1984). Evidence in support of this suggestion is reported by Egeland *et al.* on page 785 of this issue. On pages 805 and 806 the assiduous reader will find persuasive evidence from two other research groups for an inherited predisposition to manic-depressive disease that is not linked to the short arm of chromosome 11. None of these groups has actually identified the predisposing gene.

It is not in principle surprising that a defect in a single gene may cause a complex and variable disorder of human behaviour, or that the predisposition is apparently attributable to different genes in different families. It is, however, remarkable that it has been possible to establish the linkage to chromosome 11 in one of them, for reasons connected partly with the nature of the disease and partly with the more general obstacles presented by the human species to the study of genetics.

For much the same reasons, there are strict limits on the practical uses to which the new genetic markers for manic depression could be put. Manic-depressive illness (also known as bipolar affective disorder) is relatively common, affecting an estimated 0.5 to 1.0 per cent of people. Although manic-depressives characteristically undergo alternating cycles of wild excesses and profound depression, the age of onset, frequency and severity of the mood changes vary considerably, and some individuals in affected families manifest only depression. Moreover, while the effect of the gene is dominant — that is, an individual carrying only one copy can be affected — it has incomplete penetrance, which means that some fraction of those carrying it will never develop the disorder.

Genetic analysis of such a disease faces obvious difficulties. First, the diagnosis of the disorder necessarily requires subjective assessment; second, if more than one gene is involved, genetic evidence from different families cannot necessarily be pooled; and third, incomplete penetrance may make it very difficult to establish a clear link between the inheritance of a given gene and the inheritance of the disease. In any case, the unequivocal establishment of such linkages requires large and generally rather inbred families, which are rare in Europe and North America.

These last conditions are however met by the Old Order Amish whose religious convictions moreover forbid alcohol and drugs, thus eliminating one serious obstacle to the accurate ascertainment of psychiatric disease. What Egeland *et al.* have been able to establish, in brief, is that in one large Amish family, two genes known to lie close together on chromosome 11 are frequently inherited together with affective disorder, which in their study was rather stringently defined and independently diagnosed by five independent assessors.

They were able to establish this linkage because the cloned genes they used as markers lie adjacent to highly variable regions of DNA, making it extremely unlikely that a given individual will inherit identical variants of this region from both parents. By tracing the inheritance of different variants of the two marker genes, and of affective disorder, through the affected family, Egeland and colleagues were able to show that a gene predisposing to manic depression lies close to the two marker genes on chromosome 11.

What are the implications of this linkage, especially in the light of the evidence of the other two groups, Hodgkinson *et al.* and Detera-Wadleigh *et al.*, that in three Icelandic families and three North American families respectively the chromosome 11 markers used by Egeland and collaborators are not associated with affective disorder? First, although mistakes in diagnosis could more easily lead to a false negative conclusion than a false positive, all three research groups used the same standard diagnostic criteria and the negative evidence is quite convincing. This means there are at least two different genes predisposing to affective disorder.

In fact there are probably at least three: a large Israeli survey using traditional genetic markers provides strong support for earlier suggestions that some cases may be due to a gene on the X chromosome (M. Baron *et al. Nature*, in the press). Although it seems quite likely that most cases of manic-depressive disorder arise from a genetic predisposition, it is clearly impossible to tell at this stage how many will be linked to chromosome 11. Genetic heterogeneity alone will thus limit the usefulness of the chromosome-11 markers in identifying individuals at risk in the general population. But in any case the use of linked markers for this purpose is generally impossible without unusually extensive family data, as the particular variant of each marker that is linked to the disease will differ from one family to another. Moreover because of the incomplete penetrance of the gene (Egeland *et al.* estimate maximum penetrance at 63 per cent), there is no guarantee that an individual carrying the predisposing gene will ever develop the disorder.

Finally, what kind of single gene might produce the spectrum of complex disorders that seem to arise in affected families? Egeland *et al.* point out the highly suggestive recent finding that their two marker genes on chromosome 11 are closely linked to the gene encoding tyrosine hydroxylase, which is the principal enzyme in the synthesis of the catecholamines. The catecholamines comprise a major class of neurotransmitters, disturbances in whose function are implicated in a wide range of mental disorders.

It is not difficult to imagine ways in which abnormalities in the regulation of either the enzyme or the gene encoding it might lead to imbalances in neural activity that could, by inducing delayed compensatory changes in other systems, result eventually in periodic oscillations expressed as manic-depressive cycles. Nor is it hard to see how such regulatory defects might occur either through mutations in the tyrosine hydroxylase gene itself or in genes encoding (perhaps) tissue specific regulators of the gene or the enzyme.

At this stage, so little is known about either the gene or the development and regulation of neural-signalling systems as to leave the imagination dangerously unfettered. However, Moss *et al.* (*Nucleic Acids Res.* **14**, 9927; 1986) have recently reported variable regions of DNA in the vicinity of the tyrosine hydroxylase gene. Hodgkinson *et al.* used the variation to look for linkage with manic-depression in the Icelandic families and drew a blank. It is a very safe bet that a similar investigation will soon be reported in the Amish; and that the genomic organization and regulation of the tyrosine hydroxylase gene will not be left to the imagination for long.

Miranda Robertson

Solid-state physics

Liquid-nitrogen-temperature superconductors arrive

John Maddox

EVEN before last week's issue of *Nature* had been mailed to subscribers, last week's record high temperature for the transition to superconductivity, given by Strongin, Welch and Davenport as roughly 70 K (*Nature* **325**, 664; 1987), had been broken. On Monday, the *New York Times* announced that C.W. Chu and his associates at the University of Houston, last week's record-holders, had succeeded in preparing a ternary oxide that is superconducting at 98 K, well above the boiling point of liquid nitrogen at 77 K. One excited physicist wondered whether the US Department of Energy had not been overhasty in agreeing to build a proton–proton collider using helium-cooled magnets.

These developments are every bit as important as Strongin, Welch and Davenport explained last week, when they also warned that it will be some time before these materials find their way into quite small devices, let alone into the power-carrying circuits of the big machines. During the past few months in which groups in the United States and Japan have been racing toward the goal of a liquid-nitrogen superconductor, they have had to make the best they can of specimens a few millimetres across which vary markedly in their properties from one to another.

Chu's technique (see Chu, C.W. *et al. Phys. Rev. Lett.* **58**, 405; 1987) requires that a mixture of the oxides of lanthanum, of calcium, barium or strontium, and of copper should be heated for hours on end at 900 °C and that the solid mass produced should be ground and sintered at 1,200 °C for another spell. There is no assurance that the resulting specimens are uniform solids, let alone regular crystals. Indeed, it has been possible to show that many of them are mixtures of two phases, each with a different crystal structure. Most specimens appear to be replete with empty voids, hardly suggestive of the purity and order that might be thought necessary for a superconductor. One of the goals ahead is the preparation of these materials in something like a well-ordered form.

What are they? And by what stretch of the imagination can an oxide be a superconductor? In reality, the ternary oxides (or sulphides, for that matter) are more often semiconductors, which is credible enough. But one of the surprises of the past five years or so is that small changes of physical conditions or of chemical composition can profoundly change the nature of a material. Some may be ferromagnets at low temperatures and semiconductors above some critical point. Ferroelectric characteristics are common.

The oxides in the forefront of people's minds in the past few months are materials that should ideally form crystals in which the two different kinds of cation (copper on the one hand and lanthanum and barium/strontium/calcium on the other) form planar sheets in each of which the atoms are linked together by oxygen atoms and which are then stacked on each other, first one kind and then the other.

Put differently, each copper ion is surrounded by a distorted octahedron of oxygen anions, giving tight binding within each copper plane but much looser binding in the direction perpendicular to the planes. The materials on which most interest has centred in recent weeks may be thought of as derived from $LaCuO_3$ or La_2CuO by the stoichiometric substitution of group II elements such as calcium for lanthanum atoms. But neither of the starting materials is a superconductor.

What seems to happen is that, especially in the short bonds between copper and oxygen atoms within the planes, bonding electrons acquire an unusual degree of delocalization, and hence mobility. On the face of things, the situation is a little like that in graphite, in which again there is a layered structure (based on a hexagonal network of carbon atoms) loaded with potentially mobile electrons — and it is no accident that there have been several attempts in the past few years to detect superconductivity in graphite at low temperatures and high pressures, all of them unsuccessful.

Part of the reason for this is that graphite is strictly a semiconductor, but one without a finite band-gap in the usual meaning of the term; instead, the density of electronic states as a function of energy tapers to a vanishingly small waist at the Fermi level, the energy of the most energetic occupied state when all the available electrons are fitted in most economically. The vanishingly small density of states at the Fermi level militates against superconductivity, dependent as that is on the interaction between mobile electrons and lattice vibrations. The electrons that matter will be those lying near the top of the occupied band, which can be excited into a conduction state by amounts of energy as small as those of lattice vibrational quanta (phonons); in graphite, the number of electrons available for conduction will be vanishingly small. Turning the argument around, the trick in making an oxide superconductor is to arrange that the density of electronic states as a function of energy is thick-waisted at the Fermi level.

The density of states at the Fermi level is not the only criterion that must be satisfied for superconductivity at high temperature to occur. It is also necessary that the electron coupling should be close, which is assured in these oxides by the proximity of neighbouring copper ions and by their capacity to exist in doubly or trebly charged form, which allows the exchange interactions to be strong. What remains to be puzzled out is why the coupling between the electron states and the lattice vibrations should be as strong as it appears to be; for that purpose, accurate crystal structures would be a boon, but they may not be easy to come by with materials made by sintering.

Several influences seem to matter in the design of the new materials. The substitution of a group II atom for lanthanum appears to be largely a way of influencing the electronic balance in the copper-carrying layers on either side, perhaps changing the electron affinity of the copper ions. In a sense, the group II elements among the lanthanums may be likened to the influence of the voltage on the grid of an old-fashioned triode valve.

Another way of changing the density of electronic states in the copper planes appears to be a stoichiometric deficiency of oxygen atoms, which explains why the properties of the oxide superconductors so far prepared appears to be sensitively influenced by the atmosphere in which they are prepared. In some cases, a reducing atmosphere will yield a superconductor whereas an oxidizing atmosphere will not; in others, the reverse is true.

So far, it seems, these empirical truths have been learned the hard way. People have prepared samples with very different compositions and have plotted selected measured quantities on diagrams in the hope that contour lines will lead them to an optimum composition. Now it may be possible to provide some explanation of what has happened. That is one problem that cries out to be tackled.

Another is that of controlling the composition of these materials more accurately than is possible by sintering at high temperatures. For even if, for the time being, oxide superconductors will be used only in small electronic devices (will the superconducting magnetometers called SQUIDs be the first outlet for them?) the sheer mechanics of production will require uniform and reproducible raw material. That is another direction in which there will have to be progress if the potential of the new development is to be realized. ☐

John Maddox is Editor of Nature.

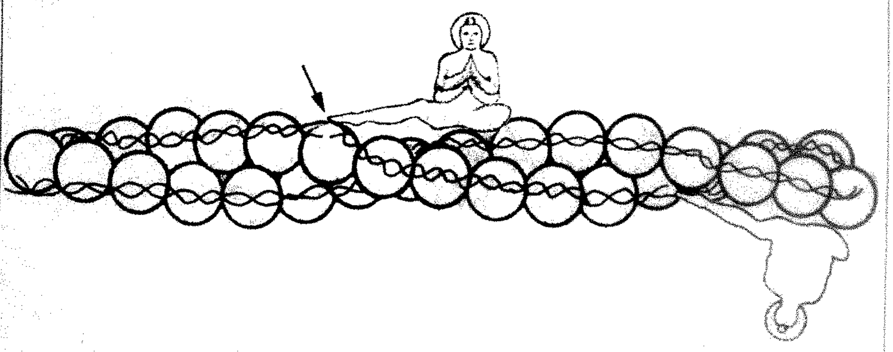
Muscle regulation

Movement on the *Aufbaubahn*

C. E. Schutt

IT IS possible that muscle evolved by near avoidance of crystallization. Perhaps, eons ago, our first motile ancestors simply salted away under some condition or other, only to be revived by the timely arrival of ATP. Indeed, the beautiful lattices in insect flight muscle reveal how closely nature, in toying with the idea of using the drive towards symmetry as a force, came to going over the brink. Electron microscopy indicates that, in the rigor state, with no available free energy, each myosin head apparently rests in peace with an actin mate on the thin filament. Thus, force develops as these proteins constantly fall towards an equilibrium association, only to be brought back again by an influx of energy-rich ATP. This cycle is regulated in vertebrate muscle by the calcium-sensitive troponin/tropomyosin system of the thin filament which teases the myosin molecules in their relentless attempts to join with actin and release the products of ATP hydrolysis[1]. Two recent crystallographic results[2,3], one of which is reported by White *et al.* on page 826 of this issue[3], and some earlier biochemical studies[4,5], point to a new way of thinking about cooperative molecular movements associated with this process (see figure).

A curious aspect of tropomyosin structure, first revealed by pattern analysis of its newly determined sequence, is a 14-fold periodicity in the position of charged and nonpolar residues on the surface of a two-stranded alpha-helical coiled-coil[6,7]. Because seven sites were expected on the basis of its alignment with actin monomers along the thin filament, these observations naturally supported the prevailing two-state on/off model for regulation, where the long tropomyosin molecule shifts *en bloc* to a new position on the actin helix when troponin binds calcium[8]. In this view, myosin heads either had a shot at an actin molecule or were blocked. Clearly, a more chemical understanding of this 'steric-blocking' mechanism would require a nearly impossible feat of crystallization to capture all the molecules *in flagrante delicto*. (The 'dream' crystal would have fourteen helically arranged actin monomers, bound to a tropomyosin/troponin complex on each side, with a complement of myosin heads thrown in.) Nevertheless it is remarkable that nearly all the principal actors in the contractile apparatus have been separately crystallized: actin[9,10]; tropomyosin[2]; troponin-C[11,12]; myosin heads (S1)[13]; and, now, fragments of troponin-T complexed with tropomyosin[3].



Troponin/tropomyosin teasing myosin with a flick of the leg. Circles represent the monomers of the helical actin filament. There are 14 monomers per helical repeat and one molecule of the troponin complex, represented by the buddha, binds at each cross-over point. Tropomyosin is shown superimposed on the actin filament; each molecule ends in a forked lap joint (arrow). On calcium binding, troponin-C, the torso of the buddha, untwists (see my previous News and Views article[17] for details of this mechanism). Inhibition of the complex by troponin-I (the buddha's knee) is thus removed. Troponin-T (the opposite leg) is bound to the lap joint of tropomyosin at the amino-terminal end (see text for details of the regulation mechanism).

It might someday be feasible to construct from crystal structures of these proteins a view at atomic resolution of the force-generating event. This is an example of the *Aufbau* problem of structural biology, namely how to build up pictures of higher-order structures from crystallographic descriptions of their components. The case of sickle-cell haemoglobin fibres illustrates how various experimental approaches can be used to work up from a monomer crystal structure to a model of the higher-order structure. In fact, a key aspect in understanding that puzzle was the demonstration that sickle-cell haemoglobin monomer/monomer contacts seen in protofilaments were the same as those in the crystals[14]. Similarly, it might be that one of the two crystal forms of monomeric actin, DNase–actin[9] or profilin–actin[10], will show actin/actin contacts like those in thin filaments (but necessarily twisted axially to fit space-group symmetry). This would be a clear case of 'speeding along the *Aufbaubahn*'.

Likewise, tropomyosin crystals, those extraordinary watery meshworks of criss-crossing coiled coils, are held together by head-to-tail intermolecular contacts, lap joints, that must be very similar to those used in muscle[2]. In a tour-de-force (no pun intended) of isomorphous replacement, White *et al.*[3] were able to exploit the mostly empty (except for water) lattices to soak in large, well-characterized, proteolytic fragments of troponin-T, the tropomyosin-binding subunit of troponin. A series of difference Fourier maps from these soaked crystals clearly shows the troponin-T amino terminus binding to the tropomyosin/tropomyosin lap joint (see figure). From this result, the authors conclude that troponin-T strengthens the lap joint, thereby enhancing cooperativity among the set of linked tropomyosins wrapping slowly around the actin helix. The globular carboxy-terminal head portion of troponin-T pins tropomyosin to the edge of the actin groove at low calcium levels, preventing it from making regular contacts via the seven strong tropomyosin actin-binding sites[3]. These sites become occupied only at high calcium levels as successive myosin heads attach, inducing the fully 'potentiated' state[5]. (White *et al.* attach no particular structural significance to the second set of tropomyosin actin-binding sites found in the linear sequence.) Thus, the state of any particular stretch of regulated thin filament, in terms of sensed calcium levels, actin conformation or numbers of bound myosin heads, is telegraphed all the way down the line, consistent with recent views[4] of cooperative regulation, where single actin/myosin binding events are no longer equivalent and independent.

The tropomyosin crystallographers[2] would like to go a step further by carrying over to muscle not only the static picture afforded by the crystals but also important details of the molecular motions seen there. The rapid and non-isotropic fall-off of intensity as a function of resolution in the diffraction pattern indicates considerable movement of the coiled-coil about its mean position, the excursion being smaller at those points where the molecules are constrained by contacts with their neighbours. How would their picture of a highly flexible tropomyosin molecule fit with the notion of cooperative regulation? The largest motions are thought to occur in the unpinned state because, by analogy with tropomyosin crystals, a smaller number of constraints apply there. Thus, when the troponin switch is thrown, tropomyosin enters a continuum, riding rein on surging actin molecules at full gallop as myosin heads struggle to mount up. As more and more myosins grab hold, a regular series of actin/tropomyosin contacts is made, establishing the fully on state. This image accords well with the dynamic movements seen in fluorescently labelled actin filaments[15] and with recent time-resolved X-ray diffraction experiments on intact muscle fibres[16]. ☐

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Conservation genetics

The future of the giant panda

Stephen J. O'Brien and John A. Knight

THE giant panda has captured media attention and the fancy of westerners since its description by Pere Armand David in 1869. In recent decades breeding pairs have been presented by the Chinese government to visiting foreign dignitaries as tokens of friendship, but only 14 giant pandas reside outside China. Despite the considerable efforts of the Chinese people and government to conserve pandas in the wild, the population continues to decline and at the present rate the giant panda will become extinct perhaps as early as the twenty-first century. Last autumn Prince Philip (president of the World-Wide Fund for Nature, WWF) visited the Wolong Natural Reserve and its Conservation and Research Centre for the giant panda in the Sichuan province on the eastern edge of the Tibetan plateau in mainland China. His visit prompted a meeting at the reserve of Chinese and foreign scientists from several disciplines with one interest in common: the biology of the giant panda and its neighbours. Based on accumulated data, a draft of a plan for preservation of the species was prepared by a joint WWF-Chinese Ministry of Forestry team headed by John MacKinnon of WWF. We report here what the Prince learned from his meeting with the group.

Panda fossils have been uncovered in many locations in mainland China and South-East Asia. As recently as 2,000 years ago pandas inhabited five Chinese provinces (Hehan, Hubei, Hunan, Guizhou and Yunnan) where they do not occur today. The remaining pandas exist in six small pockets of mountainous land (about 30,000 km^2), mainly in the Sichuan province. Pandas subsist on a nearly exclusive diet of bamboo shoots, stems and leaves and live in 2,000–3,500-m-high dense alpine bamboo forests. Twenty-one species of bamboo eaten by giant pandas in China have been identified. MacKinnon and Robert de Wulf (State Univ. Ghent) recognize suitable panda habitat as bamboo forest (higher than 2,000 m) with greater than 70 per cent forest canopy, possessing at least one species of preferred bamboo. In the Wolong Natural Reserve the two preferred species of bamboo are *Sinarundinaria fangiana* (recently renamed *Bashania fangiana*), arrow bamboo which grows at higher elevations of 2,600–3,200 m, and *Fargesia spathacea*, umbrella bamboo at 1,500–2,600 m. On these criteria only 20 per cent of the present range of the panda (6,000 km^2) remains as suitable habitat.

Between 1974 and 1977 about 3,000 Chinese scientists conducted a census of the remaining wild pandas in China, based on a transect of their known range, searching for sighting, spoor (tracks) and droppings, and estimated that between 1,000 and 1,100 animals were alive. One of the largest populations was then at Wolong (130–150 animals). George Schaller (New York Zoological Society) considered the estimates to be fairly accurate based on his own observations (Schaller, G.B., Hu Jinchu, Pan Wenshi & Zu Jing *The Giant Pandas of Wolong* Univ. Chicago Press, 1985).

Baby and mother Li Li at He Tau Ping Research and Conservation Centre for the giant panda, Wolong Natural Reserve in October 1986 (Janice Martenson).

By 1983 the Chinese government had established 12 panda reserves holding about 60 per cent of the wild panda population. Hunting of pandas was outlawed and a 2-year jail sentence was established for the killing of a giant panda. At least one farmer who accidentally snared a giant panda in the Wolong Reserve was imprisoned, and the fear of such penalties has made Chinese scientists and conservationists reluctant to handle the animals, despite assurances of support by the Ministry of Forestry in the case of accidental loss. At the Wolong Reserve, a giant panda conservation, research and breeding station was completed in 1984 and 11 captive pandas are housed there.

A new census commissioned by the national Sichuan provincial and local forestry bureau showed that by autumn 1985 several populations, including that of the large Wolong reserve, had declined by as much as 30 per cent since the mid-1970s (Ken Johnson, WWF and University of Tennessee, Knoxville). Extrapolation to other reserves suggests that the final number of free-ranging pandas may be as few as 600–700 animals. The remaining six mountain range habitats seem to be further subdivided by impenetrable barriers (for example, rivers, high ridges, logging roads and clear-cut forest) which fragment the pandas into small island populations. MacKinnon and Johnson estimate that about 35 such populations exist and that most have fewer than 20 individuals. Additionally, 10–20 per cent of the isolated panda populations found in the 1970s have disappeared.

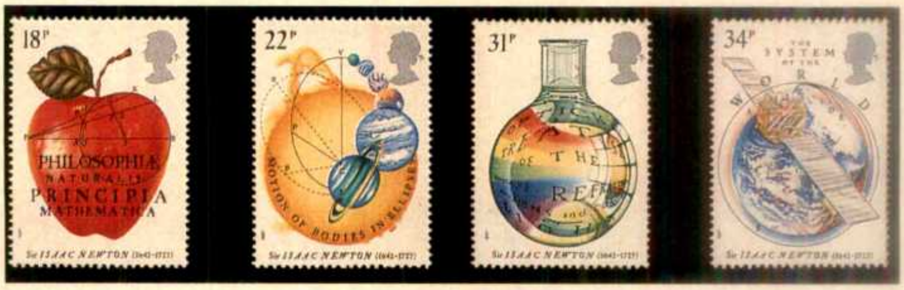
Why are numbers of free-ranging pandas declining despite government protection? There seem to be three main reasons. First, and most critical, is human encroachment. The ratio of people to pandas in China is more than one million to one, resulting in considerable pressure on the remaining sources of timber and land suitable for agriculture. Although the panda reserves contain 60 per cent of the remaining wild animals, thousands of people also live there. Giant pandas are extremely timid and will give up habitat within miles of a human settlement. Settlements in Sichuan's mountains are growing: in Wolong Reserve there are more than 3,000 people and about 100 pandas.

The second factor is forest development. The Ministry of Forestry manages the reserve both for pandas and for timber supply. Satellite photos of panda habitat in China during the past decade show a direct correlation between forest harvesting and destruction of suitable panda habitation (de Wulf). As pandas need a greater than 70 per cent forest cover over the dense bamboo, certain types of forestry operations have in many cases effectively eliminated that forest canopy.

The third factor arises from the periodic (about every 40 years) flowering and die-off of bamboo species. Although widely held to be a serious threat to the panda, it now seems that the flowering episodes are natural ecological events that the giant panda, as a species, has come to accommodate. The problem is critical only in the context of the habitat destruction and in association with the first two factors. Pandas can survive on secondary bamboo in the habitat when the principal species flowers, or if none is available they can migrate to an area where the same species has not flowered. As a result of the flowering of arrow bamboo in Wolong in 1983–85, 40–50 per cent of its pandas may have died and others may have emigrated.

The WWF plan recognizes the pressures of human development on panda habitat and outlines a programme of relief, including a reassessment of reserve boundaries. Even if the habitat were restored, however, the precarious nature of the free-ranging panda is now a numbers game. As numbers drop and small populations become isolated, two factors contribute to species demise. The first is demographic crashes: when small numbers of individuals constitute a population, the next generation may not reproduce simply because of chance effects such as accidents, altered sex ratios, snaring and bamboo flowering. A second concern is that small populations will inbreed by necessity. The genetic consequences of such 'founder effects' include inbreeding depression by expression of deleterious recessive loci that can affect reproduction, fecundity and survival. In addition, genetic homogenization by forced inbreeding removes endemic genetic variability with its evolutionary insurance against viral, bacterial and parasitic epizootics that afflict species in the wild. Overly inbred populations have a genetic 'axe' suspended over them in the form of extreme vulnerability to pathogens in the environment. These severe genetic consequences have not yet been demonstrated in wild pandas.

Captive panda Hsing Hsing at National Zoological Park, Washington DC (Jesse Cohen).



These stamps mark the 300th anniversary of the publication of Isaac Newton's book *Philosophiae Naturalis Principia Mathematica* and represent aspects of the author's work on gravitation and optics. The stamps are designed by Sarah Godwin and will be issued in the United Kingdom on 24 March. □

Conservation-genetic theory makes some predictions about small populations which are relevant to the panda's crisis. Michael Soule (*Zoo Biol.* **5**, 101–114; 1986) estimates that to have a viable population, a principal aim is to retain 90 per cent of the endemic genetic variation for more than 200 years. Empirically, animal breeders find that inbreeding depression can be avoided if the rate of inbreeding per generation remains below 1 per cent, which translates into an effective breeding size (the number of bred animals in a population) of 50. This number is a minimum estimate because the logic assumes that the starting population was sufficiently outbred initially, an unlikely assumption with pandas, some of which have a reduced amount of allozyme variations (see O'Brien, S.J. *et al. Science* **223**, 1127–1129; 1984). Further, because most of the 35 island populations have less than 20 individuals, few of the remaining panda isolates qualify as a 'viable population' in the context of predictive genetics. For this reason a possible strategy would be to re-establish the migration corridors between isolated populations to create larger panmictic wild populations. The extent of migration need not be great (less than 1 per cent per generation) to achieve enough gene-flow exchange to buffer the apparently inevitable consequences of the small population size of pandas with a very low rate of reproductive increase.

Given the low numbers of free-ranging pandas, the role of the captive breeding programme is very important to the preservation effort. Outside China, the captive population consists of about 14 animals, but inside the country there are about 100 captive giant pandas. Therefore, 10–20 per cent of the pandas live in captivity and with the rapidly diminishing wild populations, breeding these animals may effectively become pivotal in their survival. There are 137 Chinese zoos (Mr Li, Director of the Beijing Zoo), most of which want breeding pairs. The WWF management plan would improve captive breeding in conjunction with a reintroduction plan designed to reduce founder effects in the wild population.

The potential inbreeding problems perhaps caused by small founder populations become apparent from analysis of captive births of giant pandas. Of 51 captive births in China between 1963 and 1983 at the Beijing Zoo, only 19 cubs (37 per cent) survived for more than 2 months (Schaller *et al.*). This degree of infant mortality, a frequent consequence of inbreeding, is high compared with other zoo-bred species. Of the 51 births, 14 litters (64 per cent) were twins: pandas invariably raise only one member of a litter. Even if these 14 twins are considered as a single birth, the incidence of infant mortality is still 49 per cent.

Another concern is that all the recent captive litters of giant pandas in China are derived from 3 males and 10 females, an effective breeding size in captivity of 9.2 animals. By even the most optimistic of plans, the giant panda breeds worse than most species bred in captivity. But the small size of the effective breeding population may be improved by a coordinated management effort for the captive animals in China. Low fecundity may be a soluble management problem, because 8 of the 10 adults outside China have bred (Washington, Mexico City, Tokyo and a London–Madrid match). Artificial insemination has been successful in several cases and is often part of breeding programmes in China. Hence, Prince Philip's first query at Wolong was directed towards the initiation and progress of a cooperative management programme among Chinese zoological institutions.

The way forward for the giant panda must emphasize the importance of re-establishing panda habitat and implementing a coordinated captive breeding programme. There is cause for optimism because there is now a clearer perception of the problem and the solution seems attainable; most important, the Chinese people and government recognize the same hope and are determined to preserve the species. □

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Organic chemistry

Ring-current effects in C_{60}

R.B. Mallion

ONE of the most aesthetically appealing molecules to be diagnosed in recent years is buckminsterfullerene (Fig. 1), a carbon cluster — more dispassionately called icosahedral C_{60} — reported[1] two years ago by Smalley and co-workers. This apparently conjugated system (also, aptly, termed soccerballene and footballene) was independently suggested by two groups[1,2] to be the first example of a spheroidal 'aromatic' molecule; π-electron and total-energy calculations have been consistent with this idea[2-4]. Smalley's group also proposed[1] that buckminsterfullerene should exhibit strong π-electron 'ring-current' effects[5], and it is this question that is addressed by V. Elser and R.C. Haddon on page 792 of this issue[6]. These authors claim that the magnetic properties of icosahedral C_{60} are unlike those of any molecule yet encountered.

The ring-current idea of Ehrenfest, Pauling, Lonsdale and London, dating back to the 1920s and 30s, was revived with the advent of nuclear magnetic resonance (NMR) spectroscopy in the 1950s and, by the late 1960s, was the subject of considerable controversy[5]. These controversial aspects are not often referred to these days, and the ring-current concept has to a large extent been rehabilitated[7]. Using a modification[6] of London's original method that enables the Hückel molecular-orbital theory to be applied in a three-dimensional way, Elser and Haddon[6] calculate that, contrary to the expectations of Smalley's group[1], the contributions to the magnetic susceptibility of icosahedral C_{60} from the 'mobile' π-electrons in this molecule are, overall, only weakly diamagnetic.

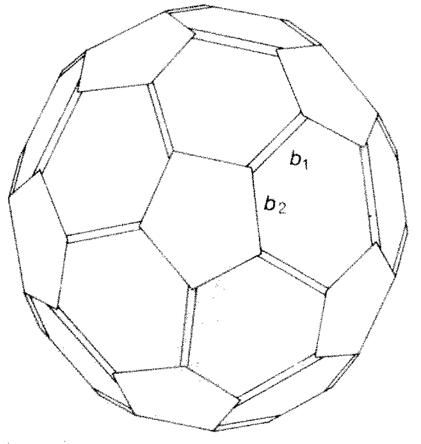

Fig. 1 Buckminsterfullerene (icosahedral C_{60} carbon cluster) and the two types of non-equivalent carbon–carbon bonds featuring in it: b_1 (shared between two six-membered rings); and b_2 (shared between a five- and a six-membered ring).

All 60 carbon atoms in icosahedral C_{60} are equivalent by symmetry, but there are two distinct types of bonds: those shared by two six-membered rings (denoted by the symbol b_1 in Fig. 1) and those situated in both a five-membered and a six-membered ring (b_2). If β_1 and β_2 represent the respective resonance-integrals appropriate to these two types of bonds, then, from bond-length considerations, the ratio β_1/β_2 $(=k)$ should be greater than one. On what they concede is the unlikely assumption that $\beta_1 = \beta_2$, Elser and Haddon calculate that the overall ring-current magnetic susceptibility of icosahedral C_{60} is only about one-fifth that of benzene and, furthermore, it is paramagnetic, rather than diamagnetic.

In these calculations, Elser and Haddon take account of the fact that, in experimental measurements of this kind, the direction of the external magnetic field is fixed in laboratory coordinates, whereas the molecule in solution is moving randomly. The NMR chemical shift (or magnetic susceptibility) is, of course, a tensor quantity, but because a molecule in solution is subject to this random motion, what is actually measured is a scalar quantity, averaged over all orientations of the molecule with respect to the applied, external magnetic field. The required average is the arithmetic mean of the three principal elements of the tensor. For benzene, this amounts to taking one-third of the 'static' value (calculated with the external magnetic field assumed to be normal to the plane of the molecule); buckminsterfullerene is, however, isotropic, and the rotationally averaged value is therefore identical with the static value.

Elser and Haddon point out[6] that the assumption of equal resonance-integrals for the two types of bonds (that is, $\beta_1 = \beta_2$, implying that $k = 1$) is unrealistic; it is much more likely that $1.0 < k < 1.3$. But they show that, as the value assumed for k is varied, the π-electron ring-current magnetic susceptibility of buckminsterfullerene, predicted to be weakly paramagnetic for $k = 1$, changes sign near k values of 1.02. It is likely that k is considerably more than this and hence the authors conclude that because just a 2 per cent change in the assumed relative bond-strengths is sufficient to tip the balance between paramagnetism and diamagnetism, the π-electron ring-current contribution to the magnetic susceptibility of icosahedral C_{60} is very probably weakly diamagnetic; an atom placed at the centre of the spheroidal system is, moreover, predicted by a non-quantum-mechanical method to be deshielded to the extent of about 0.6 parts per million in a hypothetical NMR experiment[6].

Detailed examination of the contributions from individual molecular orbitals shows[6] that this overall weak diamagnetism is in fact the result of fine cancellation of large paramagnetic contributions from some orbitals, and diamagnetic ones of similar magnitude from others. Such examination also reveals that C_{60}^{6-} is predicted to be strongly diamagnetic, and C_{60}^{12-} weakly diamagnetic — exactly the opposite of the pattern encountered with graphite and most planar, conjugated hydrocarbons, the ring-current magnetic susceptibilities of which tend to be diamagnetic for the neutral molecules and strongly paramagnetic for the corresponding reduced species.

The phenomenon encountered by Elser and Haddon[6] — that the paramagnetic/diamagnetic nature of the predicted magnetic susceptibility is dependent on the values assumed for the bond resonance-integrals — has its echo in observations made by myself and collaborators[8-10] in earlier calculations on pyracylene (Fig. 2). Pyracylene, a planar hydrocarbon, has a carbon-atom skeleton formally to be found on appropriate parts of the surface of the buckminsterfullerene sphere. In molecules such as this, paramagnetic contributions will be the largest when magnetic-dipole transitions can take place between the ground-state (occupied) and excited-state (unoccupied) orbitals, and this activity will be particularly favoured when the energy separation between such orbitals is small.

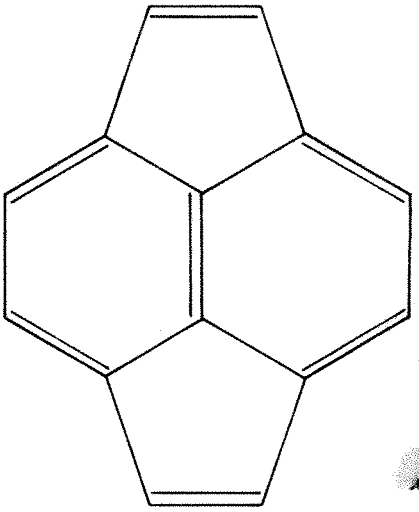

Fig. 2 Structural formula (carbon atoms only) of pyracylene, $C_{14}H_8$.

The smaller this separation turns out to be, however, the more likely is any estimated value of it to be sensitive to the peculiarities of the specific method used for its calculation. Hence, the predicted magnetic properties of such molecules are extremely dependent on the sophistication of the method (and, especially, of the wavefunction) used for their calculation.

If this (important) reservation is borne in mind, Elser and Haddon's theoretical predictions on buckminsterfullerene give a genuine impetus to future experimental work on the magnetic properties of this remarkable molecule. ☐

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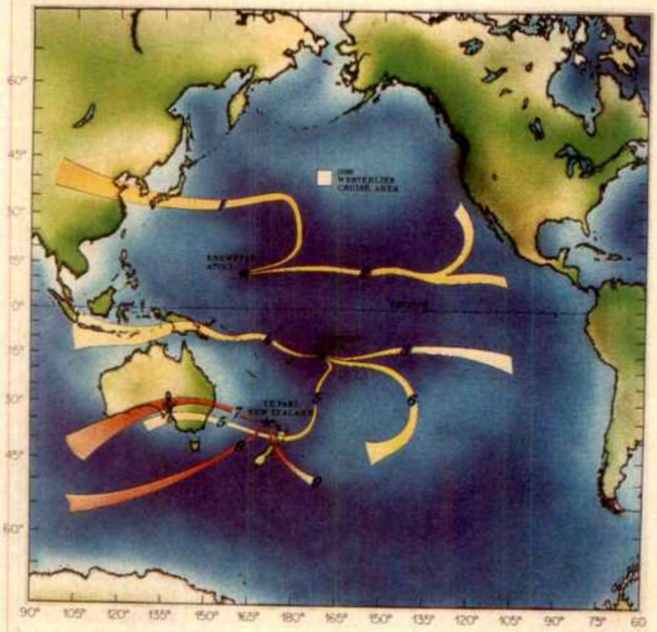
Air pollution

Tracers of atmospheric transport

Hiram Levy II

ON page 800 of this issue[1], Gagosian and colleagues combine measurements of minute quantities of plant lipids present in organic aerosols collected over New Zealand with isentropic trajectory calculations to identify specific terrestrial source regions in the Southern Hemisphere and to determine transport paths over the southern oceans. Although the sensitivity of the chemical analysis and the ability to calculate believable 10-day trajectories are impressive, the key message of the paper is the value of using a combination of chemical and meteorological tracer techniques. Taken separately, neither approach would have produced conclusive results. In combination, the assignments of source regions and transport paths are quite powerful. Therefore, it is worth asking whether such techniques, which have been used successfully in relatively remote regions to dentify distant sources, can provide quantitative information about global geochemical cycles and ultimately be applied to mportant local and regional environmental issues such as air pollution and acid rain in Europe nd North America. Before disussing this question, an examnation of some past developments may be useful.

Measurement techniques for radiouclides found on aerosols were first eveloped to assess movement of nuclear omb debris and were found to provide aluable information on atmospheric ransport. These techniques were then aplied to ^{210}Pb and ^{7}Be, the naturally occurng tracers of surface and stratospheric ir[2]. Next, filter measurements[3] in the suposedly pristine air over the Caribbean ncovered episodes of very high levels of mineral aerosol (dust). Extensive measurements[4] of dust over the remote oceans then identified large active continental sources for the material fraction of oceanic sediments. Trace metals, which were found in minute quantities on aerosols in clean air and were detected by neutron-activation analysis, as well as other sensitive analytical methods, provided another set of chemical tracers. These were used to identify the source types for background aerosols and metals such as cadmium and lead[5] and made very sensitive tracers of automobile exhaust[6]. The recent ability to measure small quantities of organic compounds found in aerosols[7] provided biomarkers that not only differentiate among terrestrial, oceanic and anthropogenic sources, but identify specific regions.



Map of the overall air mass trajectories at the major field sites of the SEAREX project, including the station used by Gagosian *et al.*[1].

In theory, the meteorological record of winds measured every 12 hours can be used to trace back in time the three-dimensional path of an air parcel, but in practice the normal meteorological data provide only the horizontal component of the wind and the record itself becomes very sparse in remote regions. Near the surface, the observed history of the horizontal component can be used to reconstruct the path of the parcel, but such trajectories are not appropriate for transport over long distances, particularly when rising and sinking motions are involved. The first successful calculations of realistic long-range trajectories circumvented the lack of measured vertical velocities by assuming that the atmospheric motions were adiabatic (no heat supplied to or withdrawn from the air parcel). Available meteorological soundings were used to construct isentropic (adiabatic) surfaces on which the air parcels moved. The resulting trajectories identified transport paths of radionuclides and ozone carried down from the stratosphere to the surface of the Earth[8]. This approximation to full three-dimensional trajectories is quite accurate for large-scale motions in relatively dry and stable air, but it does not work where there is strong mixing, such as in the boundary layer or during convection, or when there is significant latent heat release from the condensation of water. Isentropic trajectories have since been used to identify tropospheric sources; for example to track dust observed over the tropical Pacific back over 10 days to its source in arid central Asia[9] and, as reported in this issue[1], to track plant waxes observed over New Zealand.

Although previous studies have identified source regions and transport paths, they have produced little, if any, quantitative information. To do so, chemical transformations and physical processes such as aerosol coagulation and settling, dry deposition and precipitation removal must be included, either directly in the trajectory calculations or indirectly via an appropriate surrogate. Furthermore, it is not sufficient to do this for a single event. The detailed study of one or more representative transport events is important for testing a particular technique, be it chemical or meteorological, but a method is needed that integrates the results of all the events in a year or, given the year-to-year variability of weather, perhaps in a decade. As a first step, correlating surface wind direction, a primitive meteorological trajectory, with the deposition of acid rain in Bermuda clearly identifies North America as the primary source[10]. Without including chemical transformations and physical loss processes, however, the size of the North American export source cannot be determined.

The chemical tracer and isentropic trajectory methods of Gagosian and colleagues[1] seem to have some potential for quantitative studies of global geochemistry, particularly when there are a few well-separated sources and the background concentrations are low. But this type of approach is not adequate for studying regional phenomena, such as acid rain, which involve complex meteorology, chemical transformations, precipitation removal and several nearby large sources. Assigning the blame among all the neighbouring sources for acid deposition in one country or region is particularly difficult. A statistical approach using an array of chemical tracers with different signatures for different sources has been proposed by Rahn[11] and used, with some success, to apportion the sulphur fraction of acid rain in the northeastern United States among the different regional sources. However, this technique is not yet quantitative and does not work for the nitrogen fraction of acid rain. The most likely approach to pinpoint the sources of atmospheric pollution, although guided by chemical tracers, will require the development of high-resolution chemical transport models driven by realistic simulations of the meteorology of a region. ☐

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Developmental biology

Mechanism of fertilization

Brian Dale

RANKING high at present in the public eye is human *in vitro* fertilization. While politicians juggle with ethics on the use of human embryos in the laboratory, biologists have made some headway in understanding the mechanism of fertilization, using invertebrate gametes, that will help future clinical studies. Two problems of current interest are how the spermatozoon triggers the quiescent egg into metabolic activity; and why, of all the spermatozoa attached to the egg surface (Fig. 1), only one succeeds in penetrating the egg.

Work recently published[1,2] in the journal *Developmental Biology*, using techniques of electrophysiology and fluorescence microscopy to study the initial events of sperm–egg interaction in the sea urchin, have produced some unexpected results that are changing present concepts about fertilization.

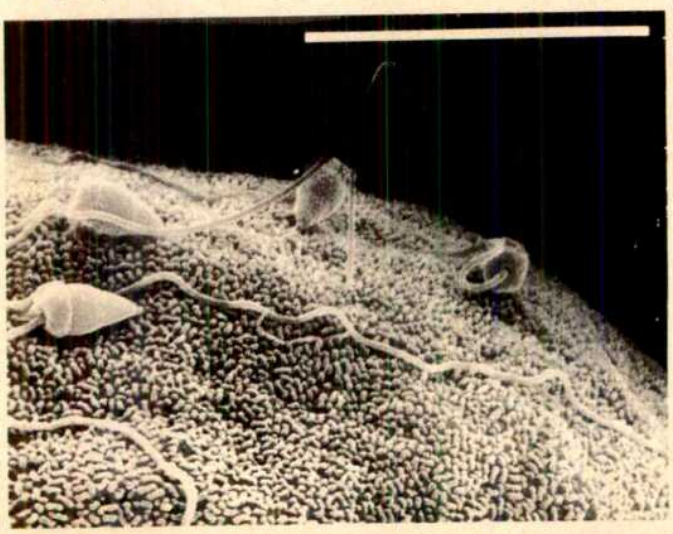

Fig. 1 The surface of a sea urchin egg a few seconds after insemination. Hundreds of spermatozoa can attach to the surface but only one succeeds in penetrating the egg. The successful spermatozoon dramatically alters the physiology of the egg within seconds. Bar, 10μm.

The first of these papers, a collaborative effort between Frank Longo and Edward Chambers's group at Miami, correlates for the first time the sequence of ultrastructural events at fertilization with changes in the electrical properties of the egg plasma membrane. In a laborious study, the authors voltage-clamped eggs, fertilized them and then serially sectioned them to locate the fertilizing spermatozoon. The moment when sperm attach to the egg was called time 0, and the first electrical event was detected 2 seconds later. Gamete fusion occurred at 7 seconds and cortical exocytosis, which leads to the elevation of a protective extracellular coat (see Fig. 2), was seen at 9 seconds. A second, larger change in conductance, indicating the global transmission of the activating impulse, was detected at 13 seconds. Chambers and McCulloch have taken the work further[3], using the electrical measurement of capacitance to time sperm–egg fusion, thereby reducing the time delay inherent in the fixation of biological material. It now seems[4] that the initial electrical event occurs simultaneously with sperm–egg fusion and that in fact it may be the result of the ion channels in the sperm plasma membrane being incorporated into the egg plasma membrane.

In the second of the two recent papers[2], a group at Miami, led by Robert Hinkley, loaded sea urchin eggs with a fluorochrome and then fertilized them. When cytoplasmic continuity between the two gametes is established the dye flows into the spermatozoon, which then fluoresces. This ingenious way of timing sperm–egg fusion not only supports the results of Chambers and colleagues, but shows that when the egg membrane potential is voltage-clamped at a negative potential, only one sperm fuses with the egg. This is unexpected, because it was thought by many that the positive shift in membrane potential at fertilization is a fast block to polyspermy[5]. Preventing voltage change by clamping should have resulted in multiple sperm fusions and entries.

There are two generally accepted ideas about how the spermatozoon triggers the dormant egg, a cell one million times its own volume, into metabolic activity. One[6] is that binding of the spermatozoon to externally located receptors on the egg surface is the primary signal, which is then transduced across the membrane to the cell interior, mobilizing second messengers such as inositol trisphosphate and calcium ions. The other[7] is that the spermatozoon contains a factor, soluble in the egg cytoplasm, that is released into the egg following gamete fusion, and which directly triggers the mobilization of secondary messengers. If the initial electrical event is the result of the spermatozoon fusing with the egg, as now suggested by Chambers and colleagues, it seems less likely that an externally located receptor mechanism is responsible for egg activation. The idea that spermatozoa contain a soluble activation factor is not new: it was popular more than 70 years ago, before the concepts of cell surface receptors were conceived. This is not to say, of course, that surface receptors are not important in fertilization, but rather their role is in the recognition and binding of the gametes, not in physiological activation of the egg.

One of the most astounding facts to an observer watching the process of fertilization for the first time is why only one spermatozoon, of potentially hundreds investing the egg surface, manages to enter the egg (Fig. 1). In many animals, including mammals, eggs *in situ* do not usually encounter large numbers of sperm, and in others the extracellular coats around the egg form a physical barrier. Nonetheless, in the laboratory, eggs challenged by hundreds of spermatozoa are usually monospermic. Several seconds after insemination, eggs from most animals undergo a complete structural reorganization of their surface, called the cortical reaction, that makes the egg refractory to further insemination (Fig. 2). But the cortical reaction is a slow propagating wave that requires many seconds or minutes, depending on the species, to spread over the surface. Thus the mechanism of fertilization must allow one sperm to interact with the egg and block the progression of supernumerary sperm within a second. An electrical fast block was considered appropriate until recently, when voltage-clamp and current-clamp experiments[8,9] cast doubt on the hypothesis. If a positive voltage prevents polyspermy, then eggs kept at negative voltages during fertilization should be polyspermic. As confirmed elegantly by Hinkley and colleagues[2], this is not so and, therefore, although voltage may be involved in sperm–egg fusion, it does not explain prevention of polyspermy. It will not be easy to think of another mechanism that changes the surface properties of a 100-μm diameter cell in fractions of a second. The only other explanation at present is that the egg surface is not homogeneous as previously thought, but contains hotspots, pre-determined sperm-entry sites.

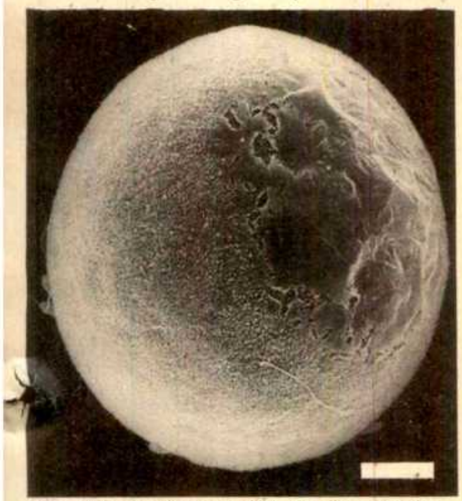

Fig. 2 The cortical reaction, the first morphological sign of activation, which involves the elevation of an extracellular coat around the egg. There, the coat has started to elevate at the top right-hand-side of the egg and will take a further 20 seconds to spread completely around the surface. Bar, 10 μm.

Research on human material is necessary to sustain and eventually to improve the success rate of a now-established treatment for some forms of sterility, and techniques to approach some of the major problems are available. Micro-injection of spermatozoa into close proximity to the egg plasma membrane, for example, may be instrumental in cases of oligospermy (low sperm count) and unexplained infertility, and electrophysiological recording will help to classify stages of egg maturity and embryo quality. Progress in this field will undoubtedly benefit from what is known and what can be learnt from the gametes of invertebrates. ☐

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Protein stabilization

Thermodynamics of unfolding

Walter Kauzmann

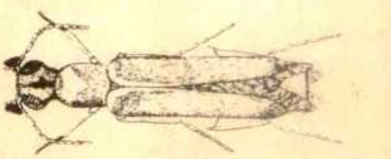
IT IS widely believed that a major factor in maintaining the specific structures of native proteins is the tendency of non-polar side chains (such as those of the leucines, valine and phenylalanine) to pack together in the interior of the protein molecule where they can avoid contact with water. Thus, proteins are stabilized by the same physical forces as those that keep oil and water from mixing — a concept called the 'hydrophobic interaction'. There is, however, some controversy over the best way in which to approach this phenomenon. A recent paper by Robert Baldwin (*Proc. natn. Acad. Sci. U.S.A.* **83,** 8069–8072; 1986) shows, somewhat surprisingly, that the simplest model system of all, provided by data on the solubility of simple liquid hydrocarbons in water, can account quantitatively for some interesting regularities contained in the mass of thermodynamic data on protein-unfolding equilibria provided by the Soviet calorimetrist P.L. Privalov and others (see Privalov, P.L. *Adv. Protein Chem.* **33,** 167–240; 1979 for a review).

One of the most striking features of the thermodynamics of the unfolding of native proteins (a process that used to be called protein denaturation) is the existence of an enormous difference in the heat capacities of the folded (native) and unfolded (denatured) states. This difference amounts to 0.1–0.2 cal deg^{-1} g^{-1} protein, and Privalov showed that it correlates with the content of non-polar residues in the interior of the protein in its folded state. Simple liquid hydrocarbons such as benzene, toluene and hexane also show a large increase in their apparent specific heats on solution in water. This amounts to 0.67 cal deg^{-1} g^{-1} for benzene and 1.2–1.3 cal deg^{-1}g^{-1} for pentane and hexane — roughly ten times larger than the change on protein unfolding, on a per gram basis. These numbers for hydrocarbon solubilization in water are astonishingly large and are similar to the specific heat of water, which, at 1.0 cal deg^{-1} g^{-1}, is itself one of the largest specific heats known, being approached or exceeded only by such materials as solid lithium, liquid or gaseous helium, solid, liquid or gaseous hydrogen and, perhaps the most surprising of all, liquid ammonia.

Because of the large change in the heat capacity between the folded and unfolded states, the energy and entropy changes for protein unfolding and for the solution of hydrocarbons in water are strongly temperature-dependent. For the solution of simple liquid hydrocarbons in water, the enthalpy change, ΔH, becomes equal to zero at 15–30 °C. Thus, the solubility of benzene in water passes through a minimum at 16 °C because $\Delta H = 0$ at this temperature for the transfer of a molecule of benzene from liquid benzene to water. At this temperature the slight solubility of benzene in water entirely results from a large negative value of ΔS for the transfer, so the rejection of benzene by water at 16 °C is said to be entirely entropy driven. On the other hand, Baldwin points out that for all these simple liquid hydrocarbons ΔS for the transfer vanishes at a common temperature close to 113 °C, which means that the low solubility of benzene in water near the boiling point of water is mostly enthalpy driven.

Using thermochemical data obtained by J.M. Sturtevant (*Proc. natn. Acad. Sci. U.S.A.* **74**, 2236–2240; 1977) on lysozyme, Baldwin then shows that if the ΔH and ΔS of unfolding are partitioned between hydrophobic contributions (for which ΔH

THE ship-timber beetle *Lymexylon navale*, formally a serious threat to timber and wooden ships, forms part of the



recent revision of the Lymexylidae genera (Quentin D. Wheeler, *Bulletin of the American Museum of Natural History* 183, 113–210; 1986). ☐

=0 at 22 °C and ΔS = 0 at 113 °C) and residual contributions, ΔH_{res} and ΔS_{res}, then the latter turn out to be independent of the temperature. They presumably represent some contribution from the protein molecule itself apart from the hydrophobic interaction. Furthermore, both ΔH_{res} and ΔS_{res} are large and positive, as would be expected if the unfolded protein is anything like a random coil in which the favourable packing interactions that exist in the native state have been disrupted. Even more significantly, the per residue value of ΔS_{res} turns out to be about the same for several simple globular proteins. Privalov had already shown that the plots of temperature against ΔS for unfolding per residue for several typical small proteins tend to intersect near 110 °C, which is a consequence of Baldwin's considerations.

Baldwin does not comment on the molecular basis of his observations; what he has to say is independent of that. This matter has been discussed at length by many authors and is controversial. I still believe that the Frank and Evans 'iceberg' model of 40 years ago is essentially correct: when it is transferred into water the non-polar hydrocarbon molecule induces, in the layer of water immediately surrounding it, a cage of more or less fully hydrogen-bonded water molecules (the iceberg). The large decrease in entropy that is known to accompany the transfer is accounted for by the increase in order accompanying the formation of the iceberg. As the temperature is raised the iceberg melts bit by bit — whence the large contribution to the specific heat. Indeed, this notion has been dealt with quantitatively by S.J. Gill *et al.* (*J. phys. Chem.* **89**, 3758–3761; 1985). This interpretation is also supported by molecular simulations of methane and neon in water, which invariably show a layer of structured water surrounding the non-polar solute molecule. Some of the attempts to explain these observations in other ways seem to me to be ludicrous, to say the least.

Baldwin's success with his model is remarkable in several respects. He wishes to explain the interaction with water of a non-polar group, R, in a molecule R–X, where X can be a single atom, such as a halogen (as in methyl chloride) or a group of atoms (as in ethanol or acetic acid, where X is –OH or –CO–OH). To do this he studies the behaviour of the molecule R–H in water, as if the nature of the group X makes no difference to the interaction between water and R. That this will work at all is perhaps not remarkable if X contains only a few atoms, but in Baldwin's case X is the polypeptide backbone, with enormous steric and other influences that could easily interfere with the success of the approach. Evidently, they do not.

The point has also been made that the interior of a folded protein molecule is more likely to resemble a solid than a liquid, so that it is wrong to use the liquid hydrocarbon as the analogue with the clusters of non-polar residues that are known to exist in protein interiors. Baldwin's success shows that this cannot be an important consideration in the phenomenon that concerns him here.

But there is a fly in the ointment. The liquid hydrocarbon model fails almost completely when one attempts to extend it to the effects of pressure on protein folding. Just as temperature effects on the unfolding equilibrium are determined by the enthalpy change, ΔH, of the process, so the pressure effects are determined by the volume change, ΔV, that accompanies the unfolding. Studies on several proteins clearly show that ΔV for unfolding is positive at low pressures but becomes negative at pressures above 1–2 kbar. (This shows that the compressibilities of the folded and unfolded forms must be very different, just as their heat capacities are different.) On the other hand, studies of the effect of pressure on the solubility in water of non-polar substances just as clearly show the opposite behaviour: ΔV for the transfer from non-polar to aqueous environment is negative at low pressures but becomes positive at pressures above 1–2 kbar. This failure of the hydrophobic liquid model is mentioned in passing by Baldwin, and workers in the field are probably aware of it in a general way, but the fact is that this discrepancy could well be devastating to the whole approach. Volume and enthalpy changes are equally fundamental properties of the unfolding process, and no model can be considered acceptable unless it accounts for the entire thermodynamic behaviour.

It is, of course, much easier to study the effects of temperature on systems than to study pressure effects. Thermostatic baths are relatively cheap and very easy to work with, whereas high-pressure apparatus (I refer to pressures of up to 5 kbar, which is really not very high) is expensive and awkward to use. It is easy, therefore, to sweep this discrepancy under the rug. A great deal more work has been done on temperature effects, and there are many more data to work with. But one is reminded of the story of the drunk who, one dark night, has lost his keys. He is seen looking for them under a street light. When asked where he lost them, he points across the street, where all is dark. "Why, then, are you looking for them here?" He replies, "Because there is more light here". Until more searching is done in the darkness of high-pressure studies, our understanding of the hydrophobic effect must be considered quite incomplete. ☐

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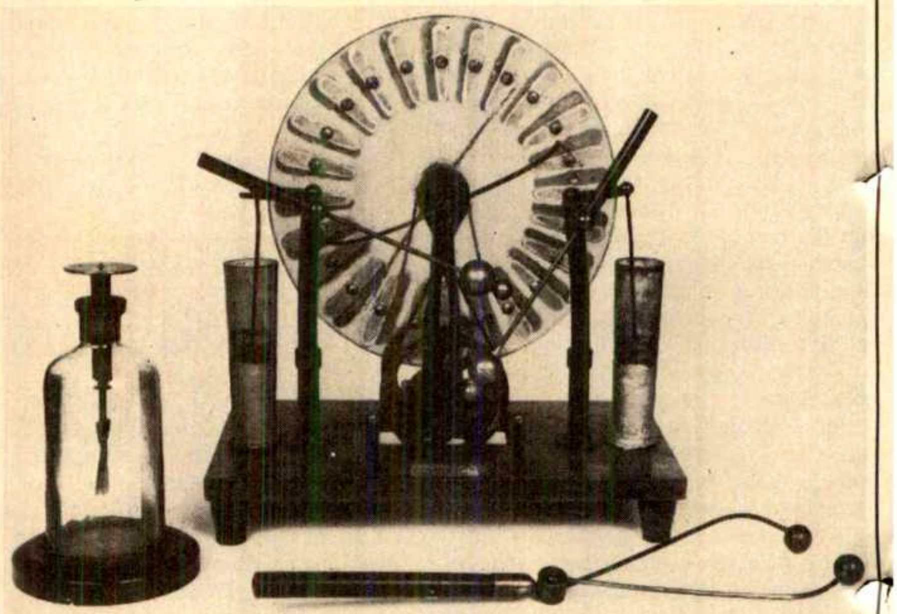

A NINETEENTH century electrostatic generator (Wimshurst pattern) by Becker & Co., with a gold-leaf electroscope and a discharging fork. The Wimshurst machine can generate thousands of volts with a few turns of the handle, producing sparks between the electrodes. These items are included in a sale of scientific and philosophical apparatus by the Wheatstone Laboratory, King's College, London, at Christie's, South Kensington, London, on 5 March at 10.30 a.m. ☐

THE picture of vortices on the cover of the 22–28 January issue of *Nature* was produced by Jim Salem (Thinking Machines Corporation, Cambridge, Massachusetts) and Stephen Wolfram (University of Illinois). ☐

The hidden dangers of AIDS vaccination

SIR—Vaccines against AIDS (aquired immune deficiency syndrome) will probably be directly tested in human immunodeficiency virus (HIV) seronegative humans before a thorough evaluation of their potential hazards is completed in animals[1] because of the shortage of chimpanzees[1,2], and the first vaccination-like programme in HIV-seropositive subjects is already being conducted in Africa[3]. But both types of experimentation may prove harmful to the volunteers even though the injected material is not infective.

Indeed, vaccination[4] and post-infection immunization[5] against other lentiviruses closely related to HIV[6-9] have induced deleterious effects. Goats injected with inactivated caprine arthritis-encephalitis virus develop more severe lesions than controls when later challenged with the live virus[4]. And the induction of a greater severity of lesions in visna virus disease by post-infection immunization[5] of sheep suggests the involvement of specific antigenic stimulation.

Autoimmune mechanisms may be involved in the production of the specific CD4 lymphopaenia of AIDS[10,11]. Therefore, we cannot exclude the possibility that AIDS vaccination in HIV-seronegative subjects may increase the severity of disease if HIV is encountered later, especially if the vaccine is not fully protective. In the same way, immune stimulation of seropositive volunteers with HIV-derived products may accelerate the disease; however, antigenic stimulation of their CD4 cells may increase the propagation of viruses to uninfected cells[10,11].

Such untoward effects are less likely to be produced by viral antigens that induce a strong neutralizing antibody response, but the ability to induce neutralizing antibodies in humans cannot be fully predicted from experiments in animals.

The expansion and prognosis of AIDS will increase the pressure to test AIDS vaccination in humans. The volunteers in such programmes should of course be fully informed about the risks they take and be able to understand this information. But the data obtained so far in animal immunization against lentiviruses closely related to HIV are a strong indication that the routine testing of the safety of vaccines in animals before use in humans should not be abandoned.

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A different perspective on coral population genetics

SIR—I wish to raise two objections to Diamond's News and Views article "Clones within a coral reef"[1]. First, Diamond misrepresents the reproductive and juvenile ecology of *Porites compressa* by equating the rarity of small (less than 60 cm^2) colonies with infrequent successful larval settlement. Low larval abundance and post-settlement mortality could also explain why small colonies are rare. There is no apparent shortage of larvae. Following one of several spawnings during a summer, the water can become cloudy with *P. compressa* eggs, and densities of 10 larvae m^{-3} sea water occur in Kaneohe Bay[2], the site of the study[3] reported by Diamond[1].

Before settlement, *P. compressa* larvae are less than 0.3 mm long[2]. A juvenile coral, under field conditions, is not visible until 2mm in diameter[4], for *P. compressa*, perhaps three months after settlement, extrapolating from growth of other species[5]. Larval settlement is also often cryptic[4-7]. Thus, only careful examination under the microscope of substrata shortly after spawning can determine if unsuccessful larval settlement or post-settlement mortality explain the scarcity of small colonies. The latter is strongly supported by studies from the Great Barrier Reef. Examinations of substrata give much higher estimates of the number of corals successfully settling than do macroscopic observations[4,6], and larval mortality is highest during the first three months after settlement[7]. Regardless of why juveniles are rare, they are important, representing new genotypes in the population.

Second, Diamond fails to discuss potential problems with using morphological traits and ultraviolet-absorbing pigments to identify genotypes. Both types of characters successfully separated genotypes in the small area of uniform depth[3] investigated in the study Diamond reports. However, *P. compressa* and other species can exhibit both changed morphology[8-10] and concentration of ultraviolet-absorbing pigments[8] when transplanted to different habitats and depths. Before morphological characters and ultraviolet-absorbing pigments are used to distinguish genotypes in more heterogeneous environments, their phenotypic plasticity must be investigated further.

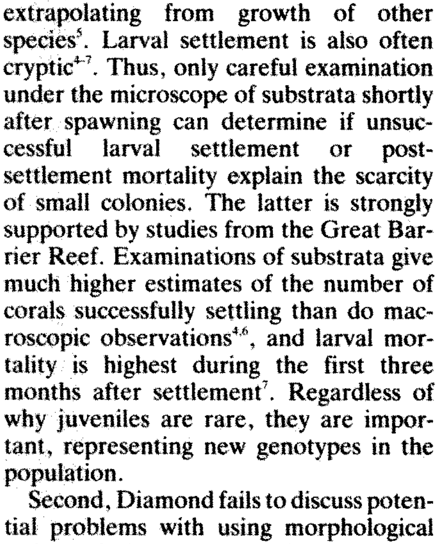

Fig. 1 *Porites compressa*, a recently settled juvenile, with the polyp retracted in the skeleton. Photographed on an experimental substratum collected from windward Coconut Island, Kaneohe Bay, Oahu, Hawaii. Maximum diameter is 0.45 mm. Juveniles of this size are not visible without magnification.

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Is there such a thing as fractal music?

SIR—Philip Campbell[1] raises the possibility of music being derived purely from mathematical formulae — an acoustic analogy to the pictorial representations of arithmetical relations, such as the iterated mappings leading to 'fractal' images (Mandelbrot sets and the like). In brief, does fractal music exist and is it interesting?

The answer to both questions is yes[2]. In fact, the fractal pieces that have been synthesized to date exhibit astounding paradoxes of perceived pitch[3-5]. One such 'composition' is a tone complex comprising many pure tones, spanning the auditory frequency range, whose adjacent frequencies are in the constant ratio $1:2^{13/12}$ (10Hz,21Hz,....,428Hz,907Hz,..... 18,284Hz). The figure shows a plot of a waveform comprising seven such frequency components. Functions such as those (imperfectly) illustrated were introduced by Karl Weierstrass into the debates on the foundations of mathematics during the last century. (Specifically, Weierstrass wanted to show his sceptical colleagues that there are functions that are everywhere continuous but nowhere differentiable. In other words, what mathematicians call a function is a lot less innocent than they were wont to believe.)

If a (computer generated) tone complex with the above frequencies is recorded on magnetic tape and played back at twice

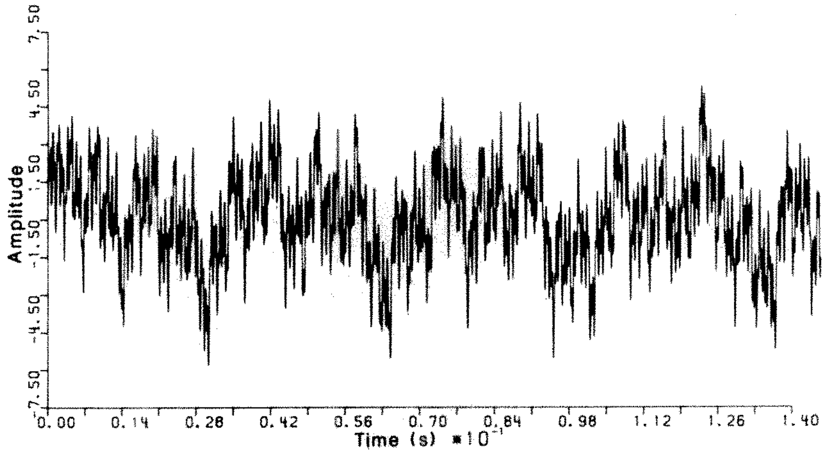

Fig. 1 Fractal waveform leading to paradoxical pitch perception.

the tape speed, it will sound a semitone lower in pitch rather than an octave higher (the usual percept engendered by tape speed doubling).

The explanation for this curious percept is based on the importance of adjacency in pitch perception[6,7]. Thus, the tone component with a frequency of 428 Hz turns into one at 856 Hz upon doubling. But in comparing the two chords, the human auditory system will identify the frequency-doubled component at 856 Hz with the nearest component in the original complex, namely 907 Hz. And 856 Hz is a semitone lower than 907 Hz. The same is true for all other components and thus a lowered pitch will be perceived.

Self-similar fractal structures are applicable not only in the frequency ('pitch') domain but can also be introduced into the time ('rhythm') domain with interesting consequences. In fact, certain self-similar number-theoretic sequences, such as the binary Morse–Thue and 'rabbit' sequences[2], give rise to very appealing rhythms.

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PHILIP CAMPBELL REPLIES — Musical quantities such as pitch and loudness have been found to exhibit 1/(frequency) type power spectra (ignoring spectral peaks due to the basic rhythms of the pieces concerned), while noise synthesized with $1/f$ characteristics sounds more 'pleasant' or 'interesting' than Brownian ($1/f^2$) or completely random noise[1]. In view of Dr Schroeder's comments it is interesting that $1/f$ noise has fractal properties. Since writing my article I have learnt that at least one composer is investigating musical design using fractals[2].

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The mechanism of chromogranin A processing

SIR—Two groups have recently conjectured that chromogranin A or a related protein may serve as a precursor for biologically active peptides in a number of cells of the diffuse neuroendocrine system[1,2], based upon the realization that pancreastatin, a peptide from porcine pancreas that inhibits insulin secretion, is closely related in sequence to residues 251–294 of bovine adrenal medullary chromogranin A[3]. What other evidence is there for this relationship?

Chromogranin A immunoreactivity in the pancreas is localized to a variety of endocrine cells of the islets of Langerhans[4-6]. The fact that pancreastatin as isolated has undergone C-terminal amidation suggests that it is produced in an intracellular, indeed intragranular, environment rather than being an artefact of tissue disruption. Enzymes which might excise pancreastatin from the chromogranin. A sequence through cleavage at single basic residues and which could catalyse its C-terminal amidation are certainly present in islets as such reactions occur in the processing of propancreatic polypeptide and proglucagon[7,8]. Chromogranin A might also be processed at any of the eight sites of dibasic amino-acid sequence as endopeptidases with this specificity also exist in islet tissue.

In the adrenal medulla, where chromogranin A was first described as a protein of relative molecular mass (M_r) 72,000, there is little indication of major proteolysis of chromogranin A apart from N-terminal signal peptide removal. The half life of the protein in these cells is comparable to that of the enkephalin precursor, being of the order of 6–8 days[9]. But a very different situation prevails in the pancreatic B-cell, where we first encountered chromogranin A as protein of M_r 21,000 (ref. 10). This protein, which we called betagranin, was cosecreted with insulin and, was shown to have an amino-acid composition and N-terminal sequence similar to bovine chromogranin A[11].

Pulse-chase labelling experiments in insulinoma cells show that betagranin is synthesized as a precursor indistinguishable in size and immunoreactivity from rat chromogranin A (M_r 80–100,000). This precursor undergoes rapid proteolysis soon after reaching the *trans* Golgi cisternae and entering the secretory granule compartment[12]. The M_r 21,000 peptide appears to be a stable end product generated by initial cleavage of the chromogranin A-like precursor at a site equivalent to Lys114 Arg115 in the bovine sequence by the action of a Ca^{2+}-dependent acidic endopeptidase[13]. The C-terminal amino acids so exposed are removed by carboxypeptidase H. As the antiserum used in these experiments is directed towards the M_r 21,000 N-terminal fragment we can say little about the fate of the rest of the precursor apart from the fact that it too is processed but at a lower rate.

On a molar basis, the estimated amount of chromogranin-related peptides in insulin granules would at most represent one per cent of the insulin content[14]. As the circulating insulin level generally lies in the nanomolar range it would take a molecule with an exceptionally long half life to achieve a blood concentration approaching the 10 nM or so at which pancreastatin was shown to exert its effect *in vitro*. This might indicate that the chromogranin A-related peptides act locally on endocrine cells or adjacent vasculature. The concept that the pancreatic B cell responds to a product of its own secretion or to other islet hormones as part of an autoregulatory cycle has frequently been invoked. It would now appear that chromogranin A-derived peptides should also be seriously considered in this context.

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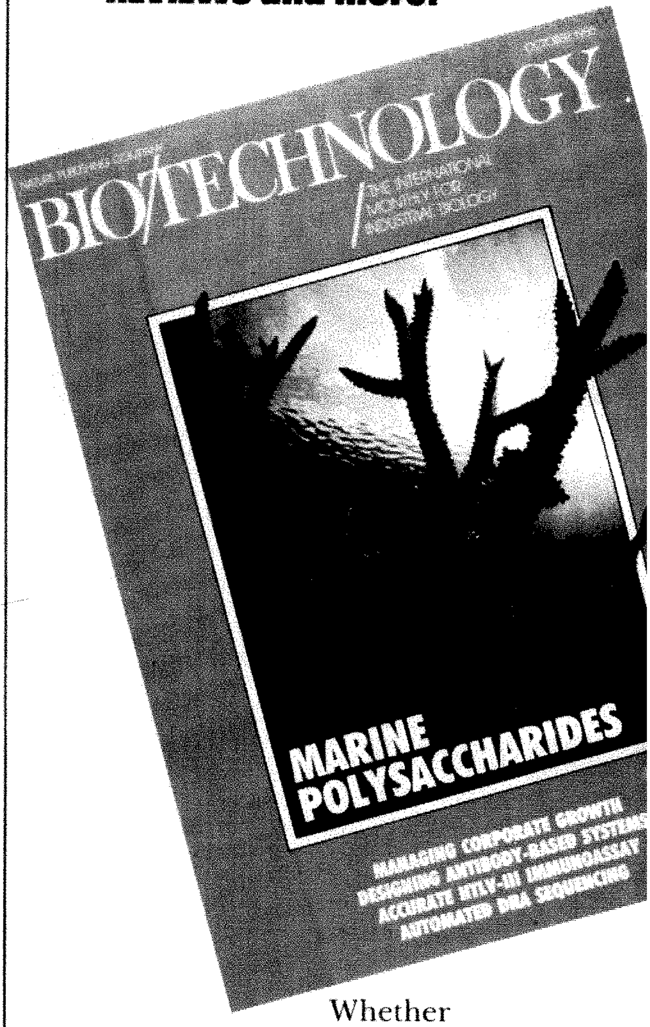
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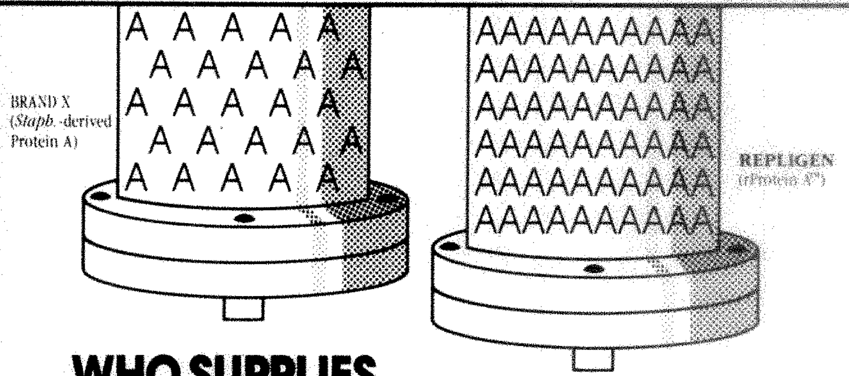

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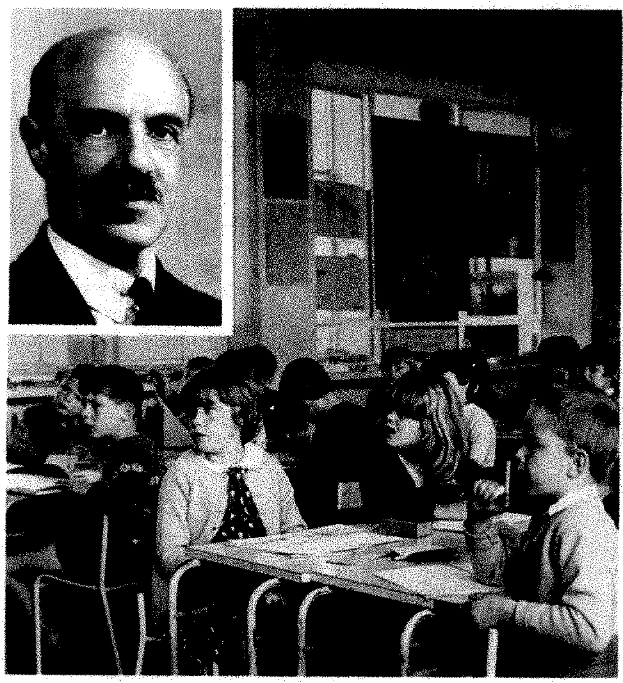
A touch of (social) class

Chris Brand

Education and Class: The Irrelevance of IQ Genetic Studies. By Michel Schiff and Richard Lewontin. *Clarendon: 1986. Pp. 243. £30, $45.*

Now that psychology has re-discovered cognition, and has lost interest in behaviourism and its laws of learning, general intelligence (g) has resumed its position as psychology's main theoretical construct — on a par with the gene for geneticists, with social class for sociologists and with the atom for chemists. This is not to say that psychologists can be relied on to accept g as originally enunciated by Charles Spearman: indeed, the aim of 'smashing' g is over-riding for many cognitive psychologists today. Yet g is certainly the concept of the widest explanatory potential known to psychology; it may possibly have a unitary biological basis — Volkmar Weiss's work in Leipzig implicates just a single gene locus as critical; and even the endeavours of cognitivists to break up g into psychological sub-components need to culminate in a story of how such sub-components can be glued together to yield the empirical observation that all measurable mental abilities are positively correlated with each other and predict socio-economic success.


Children at school — what determines their intellectual potential? Inset: Charles Spearman, originator of the concept of general intelligence.

Popperfoto/Royal Society

To date, it cannot be said that cognitive psychologists have approached g with conspicuous clear-headedness. They tend to reach for the commonsense (and faintly behaviouristic) notion that, because strategies can be found for improving performance on most mental tests, high scores achieved without special opportunities for practice and learning can themselves be attributed to brighter testees behaving . . . well, more strategically: but cognitivists fail to specify what strategy, or even what particular combination of strategies, could be relevant to the enormous range of tasks at which the higher-g subject typically enjoys an advantage.

By contrast with such vagueness, the authors of *Education and Class* are a model of theoretical lucidity. For they at least can name the variable that does the work which the London school of psychology brazenly ascribes to g itself: the name of the variable is social class, to the praise of which they devote some masterly passages of propaganda (especially their Chapter 1) and many more problematical asides.

Nor is theory alone the strongpoint of these authors. For the centrepiece of their book is a solid piece of natural experimentation (first reported in *Science* **200**, 1503–1504; 1978) which must put British social scientists and their funding agencies to shame for neglecting such opportunities for empirical enquiry. With commendable assiduity and sensitivity, the former nuclear physicist, Michel Schiff, and his French colleagues managed to trace 32 adopted children whose own biological mothers had borne other children (typically half-siblings of the adoptees) whom they had reared themselves. The essence of the study is the comparison of the intellectual and educational progress of these two, partly matched groups of children who were brought up in very different environments. The adoptees went to homes that were (on average) 1½ standard deviations high in social class (at around the 93rd percentile); and the 39 children who stayed with their own biological mothers had homes that were 1 standard deviation low in social class (around the 16th percentile). As the authors quite properly boast, no such extreme comparison of biologically matched children has previously been made.

What was the result? According to the version presented here, the adoptees had a mean IQ of 108.7, while the equivalent figure for the 'biological', non-adopted children was 94.6; and in progress at school the differences were wider. Thus are the authors emboldened to assert the causal status of social class — and to disparage other workers for their neglect of Marxist understandings of the travails of society under the artificial inequities of capitalism — and to hope that "passion can ascend through meticulous science to theoretical advance".

Sadly for Schiff and Lewontin's pleasingly simple idea of the causal importance for children of their parents' socio-economic status (SES), many qualifications are in order — some of them admitted, though with much huffing and puffing, by the authors.

(i) The apparent 14-point difference in IQ reduces to 8 points on non-verbal IQ tests, that is on those tests that would normally be used to gauge a child's true intellectual potential when its social environment had been poor. (This result appears in the French team's other publications, but not in the book.)

(ii) The parents of the adopted children would have supplied not only their high-SES homes but also the extra nurturance ("wantedness", as the authors put it) that has often been noted by other researchers to increase child IQ. Yet, despite their 'rich' environments, the adopted children were still 10 points lower in IQ than classmates at their schools who were of similarly high parental SES.

(iii) The adopted children may have been selected (for example as more alert or responsive) in infancy, and thus of greater intellectual potential.

(iv) Arthur Jensen himself has long supposed that each standard deviation of parental SES is worth (as a causal influence) some 3.35 IQ points to a child. So the adoptees in the present study, on a mainstream hereditarian account, should have had a boost of $2.5 \times 3.35 = 8.4$ IQ points (plus an extra advantage from adoption alone). Thus it is quite unclear what the authors feel they are battling against.

Further problems arise with the rest of the authors' contentions that SES is really the key variable in intellectual development and achievement.

(v) The authors discover (as did Britain's Robbins Commission in 1963) that higher parental SES boosts children's chances of entering a university, even when child IQs are matched. Yet the reverse procedure, matching children for SES and examining the influence of IQ, shows IQ to have been markedly more important than SES, as it was in Robbins's data.

(vi) The methodological problems of demonstrating the potency of SES in non-experimental data are quite unresolved by the authors: they do not even attempt path analysis. One particular source of their problems may even be that it is SES, rather than IQ, which is not readily and sensibly measurable: their own scheme assigns lower SES to antique dealers, TV announcers and farmers than to garage owners, taxi drivers and medical secretaries. Perhaps it is time for the champions of SES to do some sociometric homework?

(vii) Occasionally the authors and their contributors themselves allow that "the variance of IQ scores of children of a given social class is about 80 per cent of the total variance in the population", that "the combined genetic and non-genetic effects of social class only add up to 20 per cent of total variance in IQ scores", and even that "our methodology does not allow one to exclude the possibility of a genetic difference between social classes". Once more, such admissions make one wonder how they have managed to sustain their general enthusiasm for the endearingly simple theory of 'classism'.

The solution to this conundrum is to be found in the impassioned rejections of elitism, eugenicism and IQ in which the book abounds. Essentially, the authors' stance is that no proposition about IQ can be accepted unless it can immediately be re-written in ideologically convenient form. Yet casual re-writing can make for a wider incoherence. So a statement such as 'IQ tests are invalid and meaningless and don't reflect intelligence' is all right; but so is 'IQ tests discriminate between the social classes'. Both are ideologically attractive propositions, and possibly true: but what then, exactly, do IQ tests measure so as to yield their countless correlations? The problems of such impetuosity for the reader mount as the pages go by. If the social classes differ in IQ in some study, then the tests are invalid (in some way); if the social classes are the same in another study, then the elitist testers are exposed as forlorn incompetents. Partial correlations can be used to demonstrate the causal efficacy of SES, but certainly not to do the same for IQ. In any case, all genetic studies, even the authors' own, are irrelevant to helping the working class. Most crucially, IQ testers are held to suffer from "brutal pessimism" about the human condition: yet the authors of the present plea for State-orchestrated equalization would strip society of just those artificial products of capitalism, the advantaged families that alone seem capable of providing 14- (or 8-) IQ-point boosts to the small proportion of working class children that could ever — even under the most Maoist arrangements — be adopted by them.

Amidst the confusion, some perfectly reasonable points are made (especially in Chapter 5) in objection to the more preposterous straw men that can be made to lumber forward from hereditarian ranks. Yet the authors' insistence that the effect of genes depends on the environment would be more impressive if a single demonstrable example of this undoubted possibility could be produced from human psychogenetics: it is not clear why anyone should believe in 'reaction ranges' and 'interaction effects' as central to the causation of present human intellectual differences merely because such effects can be demonstrated by experimenters who can make gross alterations to the environment for batches of spring wheat. Moreover, the authors' assertion that "genes do not make eyes or brains" could be very misleading to those many social scientists and cognitive psychologists who already play down the degree to which humanity has a biological infrastructure as well as, for sure, a psychological and cultural superstructure.

Uncannily, as if sensing the need for more mystification with which to cover their erratic tracks, Schiff and Lewontin finally choose to obfuscate their historical materialism by insisting that "the human animal constructs itself". This propitious if somewhat fuzzy idea may well appeal to divines, to social psychologists and to the more desperate cognitivists; and it may serve as a reminder of Sandra Scarr's recent claims (unmentioned by these authors) that genetic influences express themselves more powerfully as growing children have more scope to select and create their own environments. Yet whether we will ever understand such 'self-construction' without also understanding even the most salient and well-attested features of our biology must be the first question for readers of this essentially flat-Earthist tract. ☐

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Stuff and sense

Robert W. Cahn

Advancing Materials Research. Edited by Peter A. Psaras and H. Dale Langford. *National Academy Press, Washington, DC: 1987. Pp.391. $47.50. In Britain distributed by Wiley, about £45.*

In October 1985, a conference was held in Washington, DC, to mark the twenty-fifth anniversary of the creation of the government-financed Materials Research Laboratories (MRLs). Initially there were three of them — at Cornell, Northwestern and Pennsylvania Universities — but many more were set up later and over the years they have collectively exerted an enormous influence on materials science research in the United States. This book is a record of the meeting.

The opening papers are by William Baker, Robert Sproull and Lyle Schwartz, two of whom were midwives to the birth. They map out the individual pressures and institutional reponses which led to the creation of the MRLs; describe their mid-stream transfer from the Advanced Research Projects Agency (ARPA) of the Defense Department to the National Science Foundation (NSF), and the strategic changes which this entailed; and finally attempt a judgement as to the success, past and present, of the whole enterprise. The interdisciplinary 'thrust groups' (or Materials Research Groups) favoured by the NSF to replace, progressively, the more substantial MRLs of earlier years, have focused on certain topics such as 'organic metals', ultralow temperatures, intercalation compounds and a whole range of novel materials, which are surveyed by Lyle Schwartz: he claims that many of these activities were made possible by the cooperative organization pioneered by ARPA and refined by the NSF.

Schwartz's bird's-eye view of the thrust groups' activities leads on to eleven magisterial surveys of particular fields; here, very effective use is made of limited space to convey the flavour of recent work in metallurgy, condensed-matter physics in relation to materials, ceramics, organic polymers, materials synthesis and processing, chemistry as applied to materials science, and several others. Several of these overviews are extremely impressive, marrying broad coverage with an emphasis on the most recent developments that have especial potential for scientific growth. Ductile ordered alloys, heavy-electron compounds, reptation of long-chain polymers and ceramic packaging of microcircuits are just a few of the topics discussed. John Cahn and Denis Gratias contribute yet another survey of quasicrystals (a mature subject, yet barely three years old). In this, the main section of the book, one has the sense that for once achievement has been as impressive as promise — the entire 270 pages can be recommended to labourers in the materials science vineyard who seek surveys of the interdisciplinary varieties grafted on to the solid, disease-resistant rootstock of the central disciplines.

A final short section, by a number of authors from many industries and universities, discusses some of the hardy perennials of science policy. Some but by no means all of this section is restricted to purely American issues. Small science versus large science and the grave problems of getting equipment and financing support staff in the MRLs and MRGs are debated here, as is the expected role of particular families of materials and processes in particular industries (near-net-shape manufacture, ceramics in heat-engines, surface modification, molecular-beam epitaxy and so on). Several contributors make points which would risk provoking cynical shrugs in Britain today: Albert Clogston, late of Bell Laboratories, insists on the crucial importance of *basic* technology in research and development laboratories, while Praveen Chaudhari, a senior research manager at IBM, declares that "an important point which cannot be taken for granted or emphasized enough is that the research enterprise of the nation requires an infrastructure that nurtures general science, or science that cannot be identified at present with any particular area of application". This comes from an industrialist, not from an academic!

Earlier in the book, Sproull emphasizes how the MRLs were based on the post-war recognition that Government contracts and grants "should provide much more scope for the contractor's imagination and discovery and more harvesting by informal agency–contractor interactions rather than by fulfilling specifications", and that the Office of Naval Research (one of the progenitors of the MRLs) favoured "task statements in general terms, with maximum opportunity for creation and discovery". In spite of the temporary setback of the Mansfield Amendment, the United States has by and large been able to keep these ideals. Certainly, it seems that Senator Mansfield has had a less baleful influence than Lord Rothschild in Britain.

For non-American readers, there are some *longueurs* in the book because it concentrates exclusively on the United States and its achievements. But the diet is by no means an unvaried one of self-congratulation. DiSalvo bewails the neglect of the creation (synthesis) of novel bulk materials and compares it with the French penchant, orchestrated by the Centre National de la Recherche Scientifique, for combining synthesis of novel materials with immediate assessment of properties, often in the same laboratory. This is one of several places in the book where the need is pressed for materials scientists to give more attention to solid-state chemistry.

This volume represents a landmark in the literature of materials science and engineering. It deserves a wide readership, not only among materials practitioners but also among specialists in science policy. ☐

Robert W. Cahn is in the Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK.

Life of the running waters

J. Green

The Ecology of River Systems. Edited by B.R. Davies and K.F. Walker. *Dr W. Junk: 1986. Pp.793. Dfl. 350, $148, £97.*

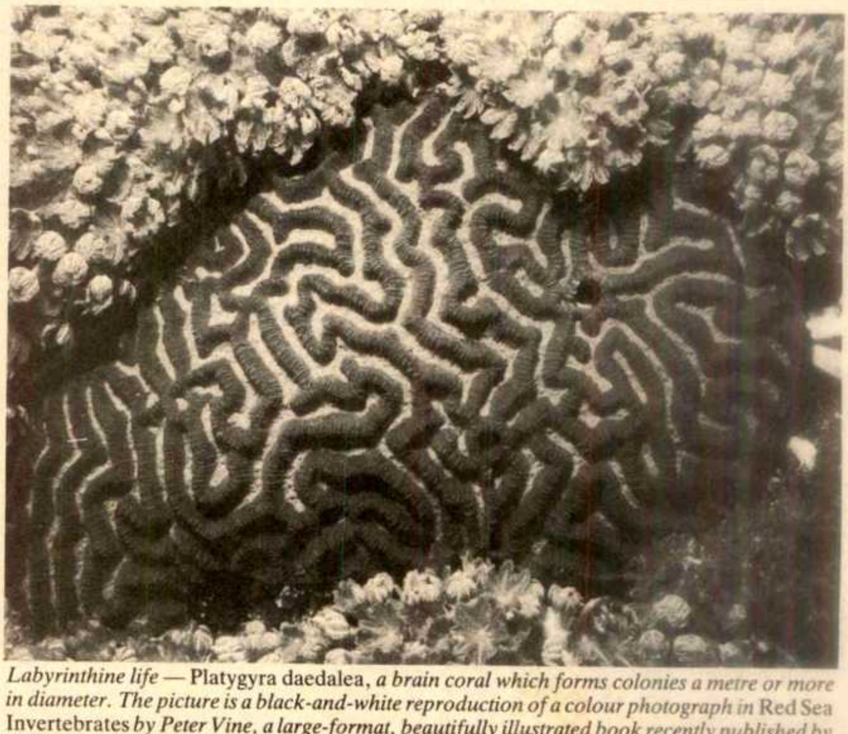
THE study of river ecology came of age only 17 years ago with the publication of Noel Hynes's *The Ecology of Running Waters*. It is most appropriate, then, that Hynes has written the foreword to the present book. Together with the introduction by the editors, he provides the integrating part of the book, distilling into a few pages the modern approach to the subject. There has been a gradual realization that a river system needs to be seen in relation to its entire catchment area — lakes, reservoirs and floodplains are essential components of any study. The emphasis throughout the book is therefore rightly on the drainage basin as a whole, and where dams have been built they are given their fair share of attention.

The main part of the book deals with the ecology of 13 major rivers outside Europe. Africa is best covered, with six, the Americas are next with five, while Australia and Asia have but one each. A huge amount of information is included, much of it not readily available elsewhere, and certainly not in the readable form that has been achieved by most of the authors.

The great value of these accounts is that they enable ecologists to make their own comparisons, between the systems described here or with some other river system on which they may be working. The gaps in each of the contributions reflect the current state of our knowledge, and by clarifying just how much, or how little, we know, the individual chapters should stimulate further research. One of the points to emerge is that for nearly half of these large rivers we do not have basic information on all the main components such as the aquatic plants, plankton, benthos and fishes. Filling these lacunae in knowledge will not be an easy matter; sampling benthos, for example, requires boats, heavy equipment and considerable manpower for sorting, and few governments are willing to give priority to such work.

From the biogeographical point of view, we require similar accounts of yet other rivers. This is not an advocation of a purely empirical approach. For our understanding of river systems to develop to a stage where generalizations and predictions may be possible, we need basic comparative data on rivers from a wide range of climatic and geological conditions. Is it too much to hope that in ten years' time we may get another book dealing, say, with the Indus, Ganges, Irrawaddy, Yangtze, Amur, Lena, Yenisei and Ob? That would form a fascinating series, and if the Mississippi and Orinoco were added we would then have, combined with the present book, the foundations for a fully scientific appreciation of some of the most vital and misused parts of our planet. ☐

J. Green is a Professor in the School of Biological Sciences, Queen Mary College, Mile End Road, London E1 4NS, UK.



F. Jack Jackson

Labyrinthine life — Platygyra daedalea, *a brain coral which forms colonies a metre or more in diameter. The picture is a black-and-white reproduction of a colour photograph in* Red Sea Invertebrates *by Peter Vine, a large-format, beautifully illustrated book recently published by IMMEL, Ely House, 37 Dover Street, London W1X 3RB, UK. Price is £39.50.*

Interacting ideas

Michael Rugg

The Brain Code: Mechanisms of Information Transfer and the Role of the Corpus Callosum. By Norman D. Cook. *Methuen: 1986. Pp.256. £30, $35.*

THE discovery of a single principle which brought order to the apparently horrendous complexities of the workings of the brain would, to put it mildly, be very welcome. The "brain code", according to Norman Cook, is such a principle, and is exemplified by the way the two cerebral hemispheres interact via their largest interconnecting fibre tract, the corpus callosum.

Racing through such topics as comparative anatomy, the evolution of intelligence, and the nature of sensory representation and processing in the cortex, Cook derives and enlarges upon his "inhibitory topographical mapping" theory of interhemispheric communication. This theory is based on the twin assumptions that the corpus callosum interconnects homotopic cortical columns, and that the fundamental mode of action of this structure (with the exception of fibres interconnecting sensory cortices) is inhibitory. Activation of a cortical column in, say, the left hemisphere gives rise to inhibition of a homotopic column on the right side, but to excitation of immediately surrounding columns because of release from "surround inhibition". The upshot is that for any pattern of cortical activation on one side, a complementary pattern (likened to a photographic negative) will be observed on the other. This principle of cortico-cortico communication is the "brain code", and is considered by Cook as likely to be as fundamental to neuropsychology as the interaction between DNA and RNA is to biology.

Despite its scope, numerous high-quality illustrations and commendably clear style, this is not a textbook; areas of controversy are frequently discussed with virtually no reference to original work, and there are many minor errors of fact and occasional howlers (as in the statement on p.162 that Wernicke's area is located in parietal, rather than temporal, cortex). The book therefore stands or falls on its theoretical contribution. This, unfortunately, stems from a highly selective review of the literature on hemisphere specialization, and a naive conceptualization of how psychological functions are represented in the brain. For example, understanding a word is assumed to occur via excitation of a single cortical column corresponding to the word's denotative meaning in the left hemisphere, and, in the right hemisphere, excitation of a population of columns surrounding the inhibited homotopic column to provide the connotative meaning. This theory is at variance both with observations of the way word meanings are lost following brain damage, and with the currently popular idea that at least some cortical areas act as parallel distributed systems in which information is represented in a spatially diffuse manner.

Cook quite correctly points to the importance of explaining how the two hemispheres interact to produce a single functional system. While criticizing others for falling back on simplistic dichotomies to characterize the different psychological functions of the two hemispheres, however, he proposes one himself, using the metaphor of an "executive" left hemisphere acting under the supervision of a "board of directors" in the right hemisphere. He further argues that the respective abilities of each hemisphere are an important factor in determining personality differences between individuals, and are amenable to psychometric measurement. Such ideas have been the subject of trenchant criticism, on both conceptual and methodological grounds, from J.G. Beaumont and colleagues (*Cog. Neuropsychol.* **1**, 191 - 212; 1984).

It seems doubtful that *The Brain Code* will achieve its highly ambitious goal. But its directness and clarity highlight our ignorance of many aspects of brain function, and will force readers to examine their own assumptions about the subject. □

Michael Rugg is a Medical Research Council Senior Research Fellow in the Department of Psychology, University of St Andrews, St Andrews KY16 9JU, UK.

Analysing the past

R.E.M. Hedges

Current Scientific Techniques in Archaeology. By P.A. Parkes. *Croom Helm, London/St Martin's Press, New York:1986. Pp.271. £30, $35.*

THE HISTORY of the application of scientific techniques to archaeological questions is a fascinating one, and starts at least as far back as Sir Humphry Davy's analysis of the mysterious pigment 'Egyptian Blue'. Ancient authorities, from Pliny on, pronounced upon its supposed composition, and recent publications from the British Museum Research Laboratory on the same material show that Sir Humphry has not had the last word.

In one sense, scientific analysis does no more than extend the judgements we all make, as between stone and metal artefacts, for example. It then falls to archaeologists to divide these objects into categories, for instance of Stone or Iron Age, or, to be more up to date, Hunters or Pastoralists. But the methods concerned have become ever-more sophisticated, while the information provided is increasingly subtle. Such information might be genetic, environmental, technological, temporal, spatial; it might not relate readily to the framework of existing archaeological thought. And the methodology itself is liable to remain a mystery to all but the scientifically initiated.

It is the latter issue, the problem inherent in the widespread use of results of advanced scientific techniques by archaeologists, that this book addresses. The perspective has been narrowed down to consideration of most of the techniques within the physical sciences that are found to be helpful at present. It will therefore be primarily of interest to those archaeologists who want to 'bone up' on the range of modern methods available. The book is organized by individual techniques — some 30 of them are included and its strength lies in this and in the generous collection of recent applications which illustrate the various methods.

Although I regret the absence of a global view, the discussion of each method is for the most part accurate and sensible; I found only a few points to quibble at in those fields I know best, and apart from the *caveat* that much of the recent literature is cited rather too uncritically, I would recommend the book for its main purpose. It also makes more attempt than most to set down simple practical knowledge for the archaeologist, for example in how to provide samples, or in its appreciation of such experimental issues as the statistical presentation of results. The last chapter on computers may help the really frightened not to run away. But although a wide range of topics is considered, the balance seems to have been determined more by the author's research interests than by the relative power of different techniques. Archaeomagnetism is treated in 38 pages (the largest section), while, for example, oxygen isotope stratigraphy receives less than three and the use of stable isotopes in the study of palaeo-diets i covered in under six.

What the book lacks above all, however, is any sense of intellectual excitement. It conveys little of the ingenuity o the scientific thought involved, or of the changing perspective of archaeological scholarship. This lack of sparkle is intensified by the dull and even tone of the writing, which I fear might widen rather than bridge the gap between the two cultures Finally, readers must surely expect bette standards of production for a book of thi very considerable price. □

R.E.M. Hedges is Director of the Radiocarbo Accelerator Unit at the Research Laboratory fo Archaeology, University of Oxford, 6 Kebl Road, Oxford OX1 3QJ, UK.

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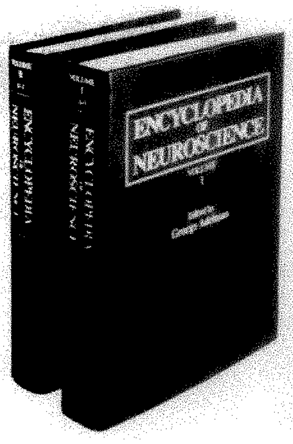

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Automated high-speed DNA sequencing

Akiyoshi Wada

The Japanese project for the development of a super-sequencer for DNA analysis has reached the point at which estimates of cost can be attempted.

THE techniques developed in the past decade for determining the base sequence of pieces of DNA have influenced the goals of molecular biology in at least two important ways. First, they have made possible a deeper exploration of the molecular mechanisms of life, as in many studies of the regulation of the activity of genes and in the localization of the genes responsible for various cryptic genetic diseases. But, second, DNA base sequencing yields data which are of interest in their own right, both in comparisons of genes with similar functions from different organisms and in the compilation, at the highest possible resolution, of the genetic maps of individual organisms. DNA base sequencing is bound to be an important part of the activity of molecular biologists for centuries to come.

As information accumulates from the sequencing of DNA from various organisms, it will become an ever more valuable asset for society as a whole, with potentially useful applications as different as a better understanding of cancer and the identification of the causes of various genetic defects and traits[1]. Unfortunately, the amount of information that will eventually have to be analysed is several orders of magnitude greater than the present rate of production of base-sequence information. Sequence data are at present being added to the databanks at the rate of 10^6 bases a year. But the human genome alone consists of 3×10^9 base pairs partitioned between 23 pairs of chromosomes, and the magnitude of the task ahead dwarfs the capabilities of the present methods.

Table 1 Estimated costs per nucleotide base, 1 Mb/day sequencer

	Cost (yen per base)
1. Sample handling, data reporting*	0.1
2. Scission, cloning, plaque selection†	0.04
3. Purification (Y500/250 bases for filter/resin)	2.0
4. Dideoxy-sequencing (kits Y100,000/100 samples)	4.0
5. Fuji Gensor gel film‡	2.0
6. Gel electrophoresis	≃0.0
7. X-ray film, processing (Y500/film)	0.5
8. Gel reading	≃0.0
9. Instrument depreciation§	0.3
10. Personnel costs¶	0.24
Total	**Y9.18**
For shotgun method (× 3)	**Y27.5**
	(In US dollars ≃ $0 .17)

Because the cost estimates of materials in items 3, 4 and 5, which represent a large portion of the total, are based on present retail prices, bulk purchase for use in a DNA sequencing factory could bring a 50 per cent reduction of the total cost, yielding 10 cents per nucleotide base.

*Assuming handling, mailing costs for one 40 kb sample of Y4,000. †M13 cloning of ≃ 500 base sequences, selection of 500 plaques yielding ≃250-base sequences. ‡Y2,500 per film, each giving 1,250 bases. §Assuming an initial cost of Y300 million depreciated over five years and operation of the sequencer for 200 days per year. ¶Assuming Y8,000 per day for each of 30 persons.

Automation

The history of human technology is that of the replacement of repetitive sequences of individually complex tasks by automatic machines. The processes of DNA base sequence analysis are essentially of this kind, and are therefore prime candidates for automation, especially now that there is general confidence in the reliability of the results of these procedures.

The potential benefits of the automation of DNA base sequencing include the relief of skilled researchers from repetitively mechanical and often tedious manipulations (which is not to say that the importance of human judgement in the interpretation of data should be undervalued) and the more rapid accumulation of data. But automation would be justifiable only if there is a substantial increase the speed of data accumulation and a reduction in the cost of analysis.

At present, one skilled researcher may, in favourable circumstances, be responsible for the sequence of up to 1,000 bases a day at a cost of something like $1 per base. It would seem that the output of an automatic DNA base sequence analyser should be some orders of magnitude greater, perhaps 1 million bases (1Mb) per day[2]. Moreover, the cost of analysis should be reduced by an order of magnitude by virtue of mass-sequencing (Table 1).

These are some of the reasons why, five years ago, the Science and Technology Council of Japan formed a committee, of which I am chairman, to study the opportunities for automation in processing DNA sequences. We have been concerned, in collaboration with industrial companies on the one hand and molecular biologists on the other, to develop the machines that could be the successive elements of a mass-production line for sequencing DNA. We have now reached the point in the planning at which all the elements of a mass production line exist, together with the interfaces between them.

In our choice of particular processes for carrying out successive chemical steps in the sequencing of DNA, we have been guided by the principles that apply in the design of engineering production lines. In particular, our choice of successive processing elements has been determined by the need for processing times at successive stages to be well matched[2]. This, for example, has led us to the use of the Sanger procedure which is much faster than the Maxam–Gilbert method for base-specific fragmentation of DNA; even so the Maxam–Gilbert method will still be important as a relief in our system for the cases for which the former method is inadequate.

We must emphasize that our goal is not that of a fully robotic system, but of a DNA sequencing line capable of yielding the sequence of 1×10^6 bases (1 Mb) per day in which skilled human operators would play a crucial part in monitoring the accuracy of the results. Although the conceptual design of our DNA sequencing line is obviously relevant to present interest in the sequencing of the human genome, we believe that there is already a sufficient need for a sequencing service to keep a system such as ours fully occupied, perhaps on an international basis.

In any event, to match the high-speed processing of such a super-sequencer, it will be necessary to establish an international DNA-sequencing centre, and to open an international channel of communication from the receipt of researchers' samples by the centre to the reporting of results of the analyses to those researchers. If such a channel is convenient and economically interesting to users, many samples will be received, increasing the number of steady users and, in turn, increasing the efficiency of analysis.

Development

A sequencing machine using the Maxam–Gilbert protocol[3,4] is now commercially available from Seiko; it can handle up to twelve samples simultaneously. For each sample, the output from this machine is a set of four groups of DNA fragments (which differ by terminating at the bases G, G or A, T or C and C respectively), which may then be subjected to electrophoresis. Unfortunately, the cycle time for this process is 12 hours, corresponding to an output of 6,000 bases a day or one base every 14.4 s, rather less than the production rate of 1 Mb a day (0.086 s per base) which is our goal.

In collaboration with Seiko, we are therefore developing a machine for automatic analysis of DNA by the Sanger method. This total system will include robotic machines for plaque selection and for extraction and purification of DNA from cosmid or shotgun libraries. From experience with a prototype constructed by Seiko under the guidance of E. Soeda of the Institute of Physical and Chemical Research at Wako, Saitama, we estimate the daily output of such a system at 300,000 bases a day, or one base every 0.28 s.

Experience shows that the resolution of the gel separations derived from the output of the Sanger machine compare favourably with those of films derived by the manual manipulation of the DNA samples. We are sure that a machine of this kind can be adapted for continuous operation, with DNA sub-samples, 250 bases or so long, supplied to the machine in accurately indexed wells in a virtually endless plastic belt. A relatively modest improvement of the capacity of this machine would achieve 1 Mb a day.

Of the key components of an automatic sequencing line, that for the electrophoretic separation of DNA fragments is the most taxing. In molecular biology laboratories, acrylamide gel films for electrophoresis are customarily prepared manually; the procedure usually requires that, after electrophoresis for between 12 and 24 hours, films should be dried and then exposed to X-ray films (where the detection of DNA fragments is based on the use of radioactively labelled bases).

In our project, Fuji Photo Film Co. of Tokyo has developed a machine for the manufacture of prepackaged acrylamide-gel films, each of which is 20 cm × 40 cm and 0.2 mm thick, with 20 pre-cast slots for sample injection at the top. Each film is covered with acetate film front (0.06 mm) and back (0.18 mm). The front covering is thin enough for the films to be read directly by exposure to X-ray film, thus obviating the need for drying. The product is now commercially available under the name of Gensor gel at a cost of US$16 per film. We hope that, by the production of films with a controlled density gradient along the direction of electrophoresis (which is within the capability of Fuji's machine), it will be possible to improve substantially on the precision with which DNA fragments may be distinguished from each other.

Fuji's pilot machine produces films at the rate of 1,000 units a day. If each film is used for the electrophoresis of five DNA sub-samples of 250 bases each in $4 \times 5 = 20$ lanes, this output is already sufficient for our goal of a production rate of 1 Mb per day. Furthermore, well-characterized and standardized films made by such a precisely controlled mass-production system will help guarantee the precision of the automation system.

We are engaged on the development of a machine in which the output of samples from a Sanger sequencer can be subjected to electrophoresis under standardized controlled conditions for periods in excess of 12 hours. Of necessity, such a machine will contain several hundred films at once. We anticipate no exceptional problems with the mechanical handling of either the gels or the X-ray films which are the ultimate products of this part of the system.

Alternatives

At least for the time being, we have based our development on the use of the conventional techniques of electrophoresis, and in particular on the use of radioactively labelled bases followed by autoradiography, but this is not to imply that we underestimate the recent development of other methods for reading and interpreting the information embodied in DNA fragments. Rather, we consider that the conventional methods are well tested, quite appropriate for parallel processing, capable of high sensitivity and accuracy and, at the same time, relatively cheap. We acknowledge that, with further development, techniques such as specific labelling of the four nucleotide bases with characteristic fluorescent dyes may be advantageous in an automated system, if their cost-performance becomes attractive.

Meanwhile, we intend that the interpretation of the autoradiography films from our system should be based on one of the automatic scanners developed independently by Hitachi Software and by Seiko. These scanners use charge-coupled device (CCD) array cameras or contact type CCD array sensors for digitizing electrophoresis signal bands. Correct recognition of the true signal on the gel, the necessary correction of deformed band shapes and the accurate elimination of spurious bands are handled by computer software.

With an autoradiograph of average quality, the error in reading is less than one per cent up to 250 bases and the maximum reading rate is 1.4 s per base ≃ 60 kb per day. Although the performance, both in reading speed and accuracy, may be a little behind that of an alert researcher, the machine can work 24 hours a day with constant precision, without training or complaining. Improvement in speed and accuracy by a factor of 10 may be attainable by using a better optical processor and a computer of higher grade. A checking system analysing the two identical (or complementary) strands by parallel analysis lines, which is the virtue of automation, may be introduced, by which the error level is reduced to the order of 10^{-4}; only patterns unidentifiable by machine are given to an expert for judgement.

Opportunity

Whether one likes it or not, we are now entering an age of mass-reading of DNA base sequences. The need for a high-performance sequencing machine is clear, and the time is ripe for its development because of progress in related fields: the accelerated speed of computation, increasingly widespread appreciation of the value of a DNA database, the development of highly sophisticated measuring devices and robotic technology and the growth of chromosome and gene libraries, especially those of humans. The advantage of a well-organized mass-analysis line is obvious, as already shown by many mass-production lines in industry; it is not only the high speed of processing and the economy of labour and cost, but also the ease of quality control and the reproducibility which ensure confidence in the result.

The significance of the large DNA sequencing centre or 'factory' should be measured not only by the current demand for such a super-machine, but also by the attractiveness of such a centre to individuals and laboratories worldwide. Such a centre would be the recipient of many samples, thus increasing the number of its regular customers and, in turn, the efficiency of its analysis. The arrangement would be self stimulating.

In the twenty-first century, we foresee that DNA-sequencing super-centres will be set up in several countries and will become symbols of the effort of nations to broaden and build on human knowledge, taking their place beside other existing symbols such as large particle accelerators, giant telescopes and far-reaching programmes of space research.

This article has emphasized the Japanese super-system; readers interested in the current status of the automation of various units in DNA sequencing are referred to the reviews by Martin and Davies[5] and by the present author[2], for more detailed discussion of equipment and for more complete references. ☐

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REVIEW ARTICLE

Strategies for antiviral therapy in AIDS

Hiroaki Mitsuya & Samuel Broder

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The retrovirus that causes AIDS has revealed enough of its life history for a variety of therapeutic strategies to be apparent. Some of these are suitable for immediate application in clinical trials or have already yielded positive results in some patients.

ACQUIRED immune deficiency syndrome (AIDS) was initially defined as the development of either an opportunistic infection or Kaposi's sarcoma (an unusual neoplasm that had previously been recognized to be associated with certain immunosuppressed states) in a person without a known cause for immunodeficiency[1,2]. It soon became apparent that these patients had a cellular immunodeficiency characterized by an inexorable depletion of helper/inducer ($T4^+$ or $CD4^+$) T cells, and within three years it had been proven that a retrovirus, which has the capacity to replicate within cells of the immune system and so lead to a profound destruction of $T4^+$ T cells, is the cause of AIDS[3,4]. The retrovirus is the third known human T-lymphotropic virus (HTLV-III). The virus has also been called lymphadenopathy-associated virus (LAV)[5] or AIDS-associated retrovirus (ARV)[6] and it has more recently been proposed that it is designated as human immunodeficiency virus (HIV)[7].

The immunodeficiency of AIDS is manifested as a loss of delayed-type cutaneous hypersensitivity reactions, a loss of certain *in vitro* T-cell proliferative responses, excessive immunoglobulin production by B cells, perhaps due to abnormal regulation coupled with a T-cell dependent mitogenic effect mediated by the virus, and a loss of *in vitro* cytotoxic T-cell responses. In addition, the opportunistic agents which most characteristically cause illness in HTLV-III infected patients (*Pneumocystis carinii*, *Mycobacterium avium* and *Candida albicans*) are normally controlled by cell-mediated (T-cell) immune responses. Once established, the immunodeficiency state is generally progressive and leads to death. The hope is that this progression can be handled and even reversed by antiviral agents that prevent replication of the virus and its cytopathogenicity in cells of the immune system.

In addition to its effects on the immune system, there is a growing recognition that HTLV-III can cause serious neurological diseases, ranging from peripheral neuropathy to fulminant dementia, with or without a clinically evident immunodeficiency[8-10]. Perhaps infected macrophages play a role in the traffic of virus across the blood-brain barrier[11,12]. Although it is still not established precisely how the virus enters the brain and how it brings about neurobiological damage, it is a virtual certainty that successful therapeutic strategies must address the consequences of, and probably prevent, viral replication in the central nervous system. Fortunately, this goal may not be out of reach for certain therapies now under study; we will return to this later.

Table 1 Stages in the replicative cycle of a pathogenic human retrovirus which may be targets for therapeutic intervention

Stage	Potential intervention
Binding to target cell	Antibodies to the virus or cell receptor
Early entry into target cell	Drugs that block fusion or interfere with retroviral uncoating
Transcription of RNA to DNA by reverse transcriptase	Reverse transcriptase inhibitors
Degradation of viral RNA in an RNA-DNA hybrid	Inhibitors of RNase H activity
Integration of DNA into host genome	Drugs which inhibit *pol* gene-mediated 'integrase' function
Expression of viral genes	'Anti-sense' constructs; inhibitors of the *tat*-III protein or *art*/*trs* protein
Viral component production and assembly	Myristylation, glycosylation and protease inhibitors or modifiers
Budding of virus	Interferons

As with any virus, the different stages in the life cycle of HTLV-III present a variety of distinct potential targets for antiviral agents. It is the purpose of this review to outline these targets, which are summarized in Table 1, and to focus on the reverse transcriptase inhibitors since there has already been some success in this area, notably with 3'-azido-3'-deoxythymidine (AZT)[13,14]. The testing of new antiretroviral agents has been facilitated by the availability of rapid and sensitive *in vitro* screening systems that determine whether a putative drug can inhibit the replication and T-cell killing activity of HTLV-III[15]. At the outset, it is worth stressing that effective therapy of HTLV-III infection may well depend on a combination of strategies that attack multiple steps in viral replication and cytopathogenicity, in part because the emergence of drug-resistant strains might be less likely.

HTLV-III as a retrovirus

In the era before the aetiology of AIDS was recognized, one of the most notable features of retroviruses was their capacity to induce neoplastic transformation in infected target cells; hence the expressions 'RNA tumour virus' and 'leukaemia virus' appeared in the literature to denote this category of virus[16]. But the virus that causes AIDS has not been shown to have a transforming capacity. (It is only linked to the causation of cancer through its immunosuppressive activity.)

By definition, retroviruses replicate through a DNA intermediate (that is, at one step of their cycle of replication, genetic information flows from RNA to DNA, a reverse or 'retro' direction[17,18]). Reverse transcriptase, the viral DNA polymerase that catalyses this step, is encoded by the *pol* gene of the virus, a gene that is conserved to a considerable extent in its amino-acid and nucleotide sequences among retroviruses[19]. These viruses have four major structural components: a core of genomic RNA; group-specific antigen (*gag*) proteins, which are involved both in the structure of the core and assembly of the virion; a lipid bilayer; and an outside envelope glycoprotein. HTLV-III is the most complex retrovirus yet characterized[20,21], in that it contains at least four genes that are not found in previously defined retroviruses (Fig. 1). The functions of two of these four genes, *sor* and 3'-*orf*, are not known. The functions of the other two genes, *tat-III*[22,23] and *art*[24] or *trs*[25], will be discussed later.

Cell binding and entry

The first step in the infection of a cell by HTLV-III is its binding to the target cell receptor. In the case of helper/inducer T cells, this receptor seems to be linked to the cell-surface protein that is recognized by T4 or CD-4 antibodies[26,27]. The process of

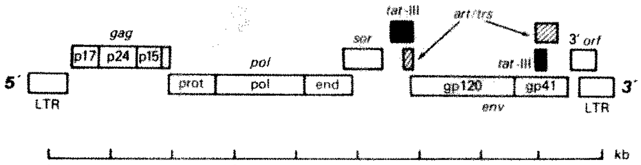

Fig. 1 Organization of the HTLV-III (human immunodeficiency virus) genome. The virus is known to contain at least seven genes, flanked at each end by long terminal repeat (LTR) segments, which contain promoter and enhancer sequences for viral transcription, as well as recognition sites for viral endonuclease (integrase) activity. Adapted from refs 20–25.

specific binding between the T4 protein and parts of the viral envelope glycoprotein may be vulnerable to attack by antibodies either to the virus or to the receptor, and theoretically certain chemicals or small peptides[28] could be designed to occupy the receptor and accomplish the same thing.

It might be worthwhile deviating from the chronology of the retroviral life cycle briefly to address the cytopathogenicity of the virus that causes AIDS. From one perspective, the T4 protein and the envelope glycoprotein of the virus determine events both at the beginning and end of the life cycle of HTLV-III within susceptible T cells. In addition to a role in the receptor-mediated entry of the virus into target T cells, the T4 protein plays a part in the susceptibility of a target T cell, once it begins to produce virus, to killing by that virus. Precisely how HTLV-III destroys T cells *in vivo* is not known. The cytopathic effect is thought to be mediated in part by an interaction between the T4 protein and the HTLV-III envelope protein that brings about lethal cell-to-cell fusions (syncytia) or a surface autofusion phenomenon that destroys the integrity of the cell membrane[29,30]. But other factors are also thought to play a role in the cytopathic effects of the virus. Recent results raise the possibility that the carboxyl terminus of the envelope protein, a region different from that which directly binds to the T4 protein, is important in the destruction of T cells by the virus[31].

Conceivably, therapeutic agents could be designed to alter the properties (for example, the lipid composition) of the surface of the virus or target cell so as to reduce the infectivity or cytopathic effects of the virus. An alternative target is the envelope protein itself. Although there is considerable variation in the protein from one viral isolate to another, the range of alterations in the binding site is most probably constrained by the need to bind to T4, which is relatively constant in structure. An antibody directed against this site might bind to (and neutralize) most strains of HTLV-III and perhaps kill infected cells as they begin to express envelope antigens, so that spread of virus to uninfected cells can be reduced.

Thus, monoclonal antibodies to the envelope protein could have a therapeutic role in patients with AIDS or related diseases. We have recently been able to produce a complement-fixing human monoclonal antibody to the major envelope glycoprotein of the first known human T-lymphotropic retrovirus (HTLV-I)[32], and the same approach could be used to develop similar human monoclonal antibodies to HTLV-III. A potential difficulty of this approach, however, is that virally infected cells might make infectious cell-to-cell contacts. Antibodies might not gain access to relevant epitopes of the envelope protein under such circumstances. Also, it has been shown that AIDS can occur in the face of what *in vitro* seem to be neutralizing antibodies to HTLV-III. Whether that is because the titres of such antibodies are low or because such antibodies do not block epitopes that mediate *in vivo* cytopathogenicity is under investigation.

After binding to a cell, HTLV-III enters the target cell by an incompletely defined mechanism, most probably a fusion process. It is conceivable that drugs could be developed to block this step, just as calmodulin antagonists block the entry of Epstein–Barr virus into B cells. Another theoretical target is the stage of 'uncoating' of the virus after it enters a target cell. In this stage, the virus loses its coating of envelope protein and its RNA is released into the cytoplasm (each virion is thought to convey a dimer of two identical genomic RNA subunits into the cell). Pharmacological agents that block viral uncoating might eventually be developed for the treatment of AIDS.

Reverse transcription

Uncoated viral RNA is used as a template for the production of DNA by reverse transcriptase. This enzyme has become a prime target for antiretroviral agents both because it should be possible to find inhibitors that will discriminate between this enzyme and the DNA polymerases of the host cell, thus avoiding side effects, and because a great deal is already known about reverse transcriptase. HTLV-III reverse transcriptase uses a lysine transfer RNA as a primer to make a minus strand DNA copy of the viral RNA as an RNA–DNA hybrid[21]. Well as having reverse transcriptase activity, the retroviral DNA polymerase possesses an inherent RNase H activity that specifically degrades the RNA of the RNA–DNA hybrid and, in theory, inhibition of this process would suppress viral replication. The reverse transcriptase then catalyses the production of a positive strand DNA, and eventually, a double-stranded DNA is formed.

The reverse transcriptase of HTLV-III has been purified and seems to exist as a p51 and a p66 molecule[33]. Large quantities of the HTLV-III reverse transcriptase should become readily available because the enzyme has been expressed in bacteria[34] and yeast (P. J. Barr and P. A. Luciw, personal communication) using recombinant DNA technology. This should aid the testing of potential reverse transcriptase inhibitors. The use of such agents in patients with AIDS is predicted on the assumption that there is continuing viral replication in the disease and that its inhibition would permit some regeneration, or at least prevent further deterioration of the immune system. Two drugs (suramin and HPA-23), which were chosen early on for clinical trials because they inhibit reverse transcriptase, have not appeared to confer clinical benefits on patients[35-37]. Nevertheless, agents which inhibit reverse transcriptase have the most likelihood of achieving an immediate clinical impact on the virus. Several promising agents that inhibit this enzyme are either already available, having been developed for the therapy of conventional viral diseases (for example, phosphonoformate[38]) or are being developed specifically for AIDS on the basis of *in vitro* screening systems for activity against HTLV-III[13,15]. We will return to one class of such inhibitors, the dideoxynucleosides, one member of which (AZT) has already been shown to prolong survival in patients with AIDS.

Integration latency and reactivation

The DNA copy of HTLV-III may be circularized during or soon after its formation, and apparently can either remain in an unintegrated form or can become integrated into the genome of the host cell. It is possible that chemicals could be developed to interfere with the viral endonuclease or 'integrase' (thought to be another function of one of the *pol* gene products) that mediates this integration step. Later, perhaps after activation of the infected cell, the DNA is transcribed to messenger RNA and viral genomic RNA by host cell RNA polymerases, and this RNA is then translated to form viral proteins, again using the biochemical apparatus of the host cell.

It has recently been shown that HTLV-III has a gene (*tat*-III) coding for a diffusible protein that markedly enhances the expression of other viral genes and viral replication. The *tat*-III gene, like the *tat* genes of the other two known human pathogenic retroviruses, is so called (by acronym) because it was originally thought to mediate a *trans*-activation of transcription—that is through a mechanism that affects the transcription of genes not in direct proximity to itself[22,23]. But ideas about the *tat*-III gene are still evolving[39,40]. The *tat*-III protein seems

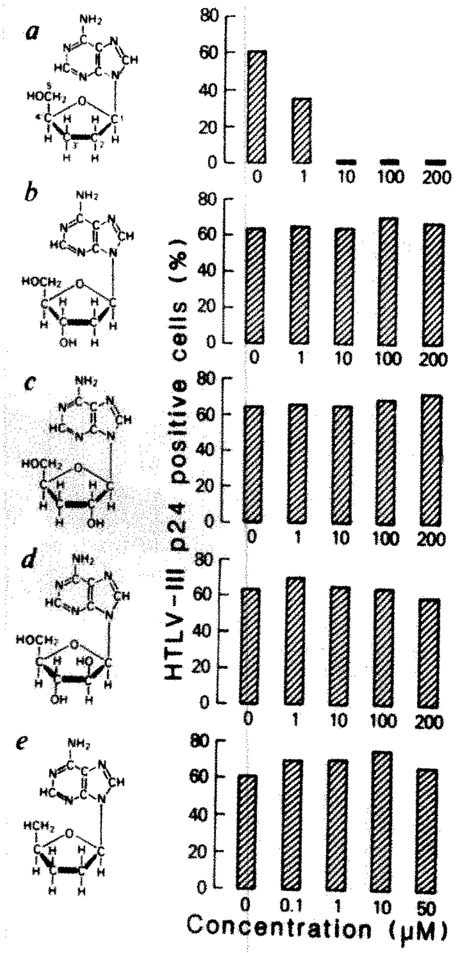

Fig. 2 Protection against infection by HTLV-III by adenosine congeners. H9 cells which are permissive for HTLV-III replication[4] were used as target cells. Cells (10^5) were pre-exposed to hexadimethrine bromide (HDB), exposed to HTLV-III_B (3,000 virus particles per cell), and resuspended in culture tubes in the presence of various concentrations of adenosine congeners: *a*, 2',3'-dideoxyadenosine; *b*, 2'-deoxyadenosine; *c*, 3'-deoxyadenosine; *d*, 9-*β*-D-arabinofuranosyladenine; *e*, 2',3',5'-trideoxyadenosine. On day 9 of culture, the percentage of cells expressing p24 *gag* protein of HTLV-III was determined. H9 cells not exposed to virus were all negative for *gag* protein. Similar results are obtained with ATH8 cells[46].

to cause an increase of viral products at a transcriptional level. However, the protein may also increase viral expression by acting beyond transcription of DNA to RNA, by enhancing mRNA stability and/or translational efficiency. Whatever the mechanism, the *tat*-III protein is thought to provide the virus with a positive feedback loop by which a viral product can amplify the production of new virions. The protein is small (86 amino acids) with a cluster of positively charged amino acids, and is thought to affect the synthesis of other proteins by binding to critical regulatory sequences at the 5'-end of viral mRNA. It may be possible to find drugs that inhibit the *tat*-III product itself, a crucial nucleic acid binding site for this protein, or both.

Employing the same mRNA that codes for *tat*-III, but using a different reading frame, the *art* or *trs* gene of HTLV-III produces a different small (116 amino acids) positively charged protein, which is thought to function as a second essential *trans*-acting factor in viral replication[24,25]. In the absence of this regulatory factor, *gag* and *env* encoded protein synthesis is severely diminished, so drugs that bind or inactivate this protein would be expected to inhibit viral replication. There is no doubt about the existence of this new regulatory gene, but the mechanism of action of its product is controversial. The action was first described as an abrogation of negative regulatory effects on translation of viral mRNA that encodes HTLV-III structural proteins (hence the name *art* for anti-repression *trans*-activator)[24]. It is possible that *tat*-III controls *art*, which in turn regulates the activity of *gag-pol* and *env*. More recently Feinberg *et al.*[25] have suggested that the gene product balances the amount of spliced and unspliced viral RNA to permit the preservation of *gag-pol* and *env* mRNA (hence the alternative name, *trs* for *trans*-acting regulator of splicing).

Although the precise mechanisms are matters of future study, it is clear that this retrovirus has evolved an astonishingly complex system of genetic regulation. Perhaps this is because of the race between viral replication within a cell and the destruction of the cell that the virus has commandeered, leaving no tolerance for inefficiency or improper timing in the synthesis of viral components. With luck, the very complexity of the virus could contribute to its defeat.

Protein production and assembly

It is conceivable that drugs that interfere with the structure and function of retroviral mRNA transcribed from integrated DNA in infected cells could be of therapeutic value in AIDS. One drug, ribavirin, is believed to act as a guanosine analogue that interferes with the 5'-capping of viral mRNA in other viral systems, and perhaps could be useful in retrovirally-induced disorders[41]. It is expected that the clinical efficacy of ribavirin in treating HTLV-III infection will be determined at the conclusion of studies now under way.

Another approach would be the use of 'anti-sense' oligodeoxynucleotides, already tested *in vitro*[42]. Basically this approach employs short sequences of DNA (or DNA that is chemically modified to enable better cell penetration and resistance to enzymatic degradation) whose base pairs are complementary to a vital segment of the viral genome. In theory such anti-sense oligodeoxynucleotides could block expression of the viral genome through a kind of hybridization arrest of translation or possibly through interference with the binding of a regulatory protein such as *tat*-III. Indeed, as the *tat*-III gene and the *art/trs* genes are expressed on the same mRNA transcript, it is possible that one anti-sense oligodeoxynucleotide could interrupt two functions that are vital for HTLV-III replication. In principle, it might be possible to achieve the same goal by constructing an 'anti-sense' virus (a retrovirus that has been genetically engineered to produce a stretch of mRNA that will bind to the messenger made by the wild-type virus).

The final stages in the replicative cycle of HTLV-III involve crucial secondary processing of certain viral proteins both by proteases (a function of one of the *pol* gene products) and by myristylating and glycosylating enzymes (provided by the host) as a prelude to assembly of infectious virions. Therefore, additional strategies for the treatment of AIDS might involve certain kinds of protease inhibitors or drugs which dampen or alter myristylation and glycosylation steps in the synthesis of viral components[43,44]. Finally, retroviruses are released by a process of viral budding, which may be inhibited by interferons[45].

We have focused the discussion on how to suppress HTLV-III, but it might be worth noting that the virus could set off a chain of secondary events (such as autoimmune reactions, the production of toxic lymphokines) that are necessary for the expression of clinical disease. It is also intriguing to speculate that a combination of antiretroviral therapy coupled with bone marrow or lymphocyte replacement therapies might be successful in certain subsets of patients with HTLV-III infections.

Dideoxynucleosides

A broad family of 2',3'-dideoxynucleoside analogues can be metabolized to become potent chain-terminating inhibitors of HTLV-III reverse transcriptase. *In vitro* these analogues can

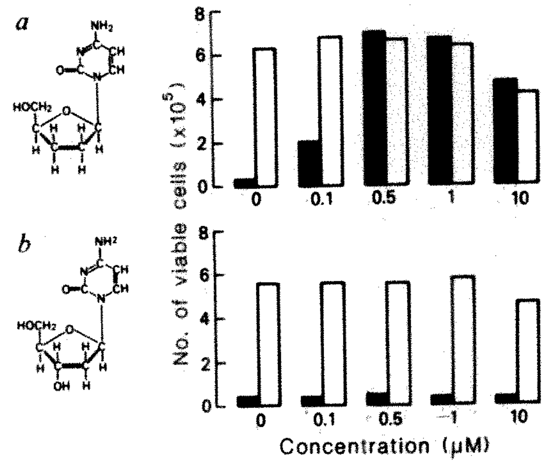

Fig. 3 Inhibition of the cytopathic effect of HTLV-III by 2',3'-dideoxycytidine. ATH8 cells[13] (2×10^5), which are sensitive to the cytopathic effect of HTLV-III, were pre-exposed to HDB, exposed to a high multiplicity of infectious HTLV-III$_B$ (2,000 virus particles per cell) in culture tubes (solid columns) in the presence of various concentrations of 2',3'-dideoxycytidine (*a*) or the normal nucleoside, 2'-deoxycytidine (*b*). Control cells (open columns) were similarly treated, but were not exposed to the virus. On day 5, total viable cells were counted. Under conditions of low multiplicity of infection, concentrations of drug ≥10 nM suppressed the virus.

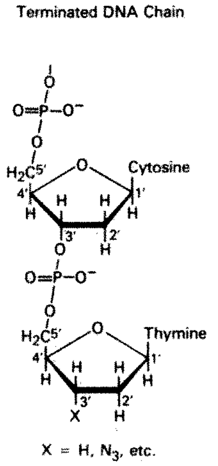

Fig. 4 Possible mechanism of activity against HTLV-III of 2',3'-dideoxynucleosides as triphosphate products. When the 3'-carbon of the deoxyribose is modified by certain substitutions (shown by X) that replace the normal 3'-OH, it is not possible to form 5'→3' phosphodiester linkages that are necessary for DNA elongation in the replication of the virus from an RNA form to a DNA form. Similar considerations apply when the sugar is in a 2',3'-didehydro-2',3'-dideoxy (double bond between the 2' and 3' carbons) configuration.

completely inhibit the replication of HTLV-III and its capacity to destroy T-cell cultures at concentrations that are 10–20 fold lower than those that impair the proliferation and survival of target cells[46]. Pioneering studies of several of these compounds have been accomplished over the past twenty years or so[47–53] and, in triphosphate form, they are familiar to molecular biologists as reagents for the Sanger DNA-sequencing procedure[53]. But their application to human antiretroviral therapy demands a new perspective. The dideoxynucleoside analogues are of special interest because they prove that a simple chemical modification of the sugar moiety can predictably convert a normal substrate for nucleic acid synthesis into a compound with a potent capacity to inhibit the replication and cytopathic effect of HTLV-III, at least *in vitro*[46].

Certain relationships between the structure and activity of adenosine analogues are explored in Fig. 2, which summarizes the capacity of five congeners of adenosine to act as inhibitors of HTLV-III. In these experiments, the adenine portion of the nucleoside analogue is kept constant whereas several simple changes in the sugar moiety are tested as variables. The reference agent is 2'-deoxyadenosine, which is a normal building block for DNA and so, as expected, has no antiretroviral activity in this system (Fig. 2*b*). But a simple reduction (removal of the oxygen) at the 3'-carbon of the sugar, creates a potent inhibitor of the retrovirus, 2',3'-dideoxyadenosine (Fig. 2*a*). A further reduction at the 5'-carbon, creating 2',3',5'-trideoxyadenosine (Fig. 2*e*) nullifies the antiretroviral effect, and neither 3'-deoxyadenosine (cordycepin) nor the *arabinosyl* derivative (Ara-A) inhibits the virus. It is important to note that these experiments were performed under conditions of high estimated multiplicity of infection[15].

We are currently developing 2',3'-dideoxycytidine as a possible agent for treating patients infected with HTLV-III. On a molar basis, dideoxycytidine is the most potent antiviral nucleoside that we have tested. Under certain *in vitro* conditions, the drug can suppress HTLV-III at concentrations of 10 nanomolar or below. This dideoxynucleoside analogue was selected for clinical development because of its *in vitro* potency against HTLV-III[46] (see Fig. 3), relative resistance to cytidine deaminase (a major catabolic enzyme for cytidine analogues)[54], good oral bioavailability, straightforward pharmacokinetic clearance by the kidney, failure to reduce normal intracellular pyrimidine pools and comparative lack of toxicity when administered to animals. We have recently begun to administer this drug to patients.

Several issues related to the anti-retroviral effects of 2',3'-dideoxynucleosides are as yet unresolved, but it seems that as they are successively phosphorylated in the cytoplasm of a target cell to yield 2',3'-dideoxynucleoside-5'-triphosphates, they become analogues of the 2'-deoxynucleoside-5'-triphosphates that are the natural substrates for cellular DNA polymerases and reverse transcriptase. (It is generally thought that nucleoside-5'-triphosphates do not cross cell membranes and are not active as drugs because of their ionic character and comparatively low lipophilicity.) Such analogues could compete with the binding of normal nucleotides to DNA polymerases, or could be incorporated into DNA and bring about DNA chain termination because normal 5'→3' phosphodiester linkages cannot be completed (see Fig. 4). At concentrations that are achievable in human cells, a dideoxynucleotide analogue can serve as a substrate for the HTLV-III reverse transcriptase, resulting in DNA chain termination after the addition of a single dideoxynucleotide residue[55].

Pyrimidine and purine dideoxynucleoside analogues can inhibit the *in vitro* replication and pathogenic effects of a range of animal and human retroviruses, even when the pathogenic effect being monitored (transformation) requires only a single round of replication; moreover, with certain lentiviruses (a family of animal retroviruses related to HTLV-III[56]), these drugs can reduce the *in vitro* viral infectivity by more than five orders of magnitude[57]. We have found that two dideoxynucleosides, 2',3'-dideoxycytidine and AZT(3'-azido-3'-deoxythymidine) can block the infectivity of HTLV-I against helper/inducer T cells *in vitro* (S. Matsushita, H.M., M. S. Reitz and S.B., unpublished data). Indeed, so far we have not observed any type or strain of retrovirus that is resistant to 2',3'-dideoxynucleoside analogues in appropriate target cells; however, the emergence of drug-resistant mutants must always be considered possible. It is important to stress that the phosphorylation reactions crucial to the activation of the nucleoside analogues are catalysed by host cell kinases, and therefore caution must be used in extrapolating from cells of one type (or species) to another.

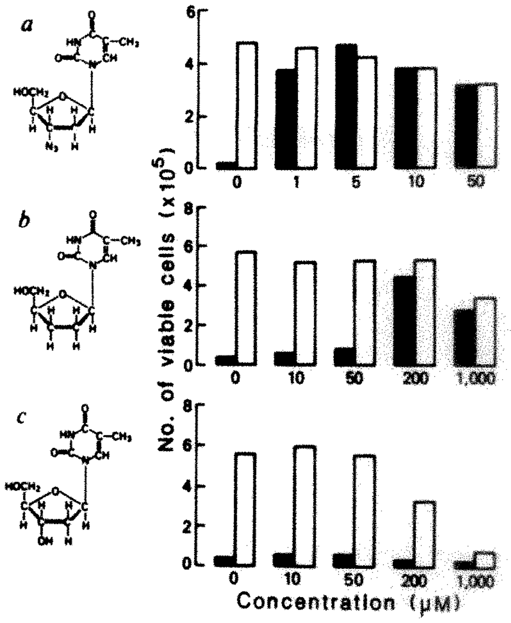

Fig. 5 Inhibition of the viral cytopathic effect by thymidine congeners. *a*, By 3'-azido-3'-deoxythymidine; *b*, by 2',3'-dideoxythymidine; *c*, by the normal nucleoside, thymidine. Experimental protocol as in Fig. 3: solid bars, virus-treated and open bars, control cells.

If these kinases are lacking in the host cell, the retrovirus will appear resistant to the nucleoside analogues, but if the retrovirus is permitted to replicate in a different target cell which has the appropriate enzymes for anabolic phosphorylation, it will appear sensitive again. Similarly, we have observed that one dideoxynucleoside (2',3'-dideoxythymidine) behaves as a relatively poor substrate inhibitor for human thymidine kinase ($K_i >$ 500 μM). The substitution of an azido group at the 3'-carbon of this analogue, yielding AZT, produces a compound that is an excellent substrate for thymidine kinase (K_m 3 μM) and is a very potent inhibitor of HTLV-III replication (Fig. 5*a*), of which more below. In this context, the lack of activity against HTLV-III that was observed using 2',3',5'-trideoxyadenosine (Fig. 2*e*) probably relates to the fact that the 5'-site is unavailable for phosphorylation. To re-emphasize, the retrovirus does not provide its own enzymes for phosphorylation; therefore, unlike herpes viruses, HTLV-III cannot adopt a strategy of mutating a kinase gene to develop drug resistance.

There are data to suggest that the HTLV-III reverse transcriptase is much more susceptible to the inhibitory effects of dideoxynucleotides as triphosphates than is mammalian DNA polymerase α (H.M., Z. Spigelman, R. P. McCaffrey and S.B., unpublished data), an enzyme that has key DNA synthetic and repair functions in the life of a cell. Indeed, reverse transcriptase has a higher affinity for dideoxynucleotides than for normal substrates. This parallels what had been learned in animal retroviral systems (see discussion in refs 46 and 58). Although 2',3'-dideoxynucleoside-5'-triphosphates can inhibit mammalian DNA polymerase β (a repair enzyme), and DNA polymerase γ (a mitochondrial enzyme)[58], we have observed that dideoxynucleosides can suppress HTLV-III replication and protect sensitive helper/inducer T-cell target cells *in vitro* for long periods without interfering with the function and survival of target T cells[55]. As discussed above, our working explanation for the activity of these drugs against pathogenic retroviruses is that following anabolism to nucleoside-5'-triphosphates, they bring about a selective chain termination as the RNA form of the virus attempts to make DNA copies of itself. The inherent RNase H activity of the viral DNA polymerase or cellular enzymes would be expected to degrade the viral RNA, giving the virus no second chance. Thus, one explanation for the selective antiretroviral activity of these compounds is that the viral enzyme is more easily fooled into accepting a dideoxynucleotide than is its mammalian counterpart. Alternatively (or additionally) the virus may have less capacity than the host cell to repair the incorporation of the false nucleotide. In some cases, however, such drugs may perturb normal nucleoside metabolism or exert other effects that result in drug-related cellular toxicity. We will return to this point in the specific context of AZT, a special congener of dideoxythymidine.

AZT

Although synthesized over twenty years ago by Horwitz *et al.*[48] and shown to inhibit C-type murine retrovirus replication *in vitro* by Ostertag *et al.* more than 12 years ago[59], no medical application of AZT emerged until recently. As shown in Fig. 5*a*, AZT is a very potent *in vitro* inhibitor of the replication of HTLV-III and its cytopathic effect in susceptible target T cells, and it has an antiretroviral effect against widely divergent strains of HTLV-III[13]. The drug undergoes anabolic phosphorylation in human T cells to a nucleoside-5'-triphosphate[60], which can compete with thymidine-5'-triphosphate (TTP) and serve as a chain-terminating inhibitor of HTLV-III reverse transcriptase.

In that sense, AZT parallels the other dideoxynucleosides. But it can also bring about several alterations of nucleoside metabolism within host cells, which may be of clinical significance. For example, thymidine kinase catalyses the formation of a monophosphate derivative of AZT, which then serves as a competitive inhibitor, with low V_{max}, of thymidylate kinase (an enzyme that catalyses the second phosphorylation step to thymidine diphosphate), leading to the reduced formation of the third phosphorylation product, thymidine triphosphate (TTP)[60]. From this perspective, AZT has an interesting capacity to lower the level of its natural substrate competitor (TTP) for viral and cellular DNA polymerases. AZT can also reduce 2'-deoxycytidine triphosphate (dCTP) levels[60], but the mechanism of this effect is unknown. How these reductions of normal pyrimidine pools might affect the balance between antiretroviral activity and drug-induced cytotoxicity also remains unknown. Nevertheless, pyrimidine starvation is likely to contribute to bone marrow suppression, one of the key side effects of the drug. This might be overcome by regimens that combine AZT with an agent that does not appear to deplete intracellular pyrimidine pools, such as dideoxycytidine. Other features which might have substantial clinical relevance, but remain unexplained at this time, include the capacity of aciclovir (a

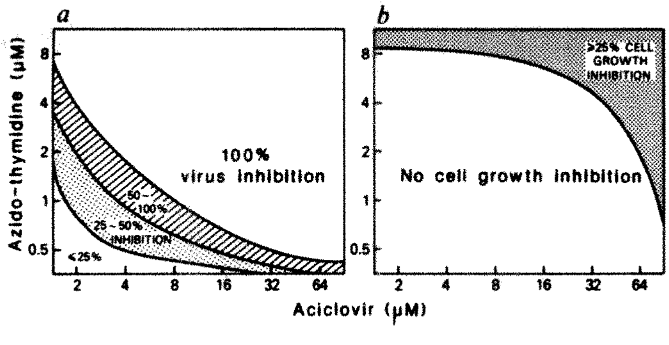

Fig. 6 Synergistic antiretroviral effect of AZT and aciclovir. ATH8 cells (2×10^5) were exposed to a very potent preparation of HTLV-III and cultured in the presence of various amounts of AZT and aciclovir. On day 6, viable cells were counted and the percentage of virus inhibition (*a*) (protective effect of the drugs on the survival of ATH8 cells exposed to the virus) and cell growth inhibition (*b*) were determined[15]. Note that although aciclovir alone showed rather weak antiretroviral effect at ≤ 64 μM, combinations of both drugs exhibited a synergistic antiretroviral effect at a wide range of concentrations without affecting the growth of cells. In *a*, cross-hatched area represents 50–100% virus inhibition; dotted area 25–50% inhibition. Dotted area in *b* denotes $\geq 25\%$ cell growth inhibition.

guanosine analogue with potent activity against herpes viruses but rather weak antiretroviral activity) to potentiate the antiretroviral effect of AZT *in vitro* (Fig. 6).

We began administering AZT to patients with AIDS and related conditions in a phase I (feasibility and toxicity-testing) trial in July 1985. That study showed that the drug had good oral bioavailability and excellent penetration across the blood-brain barrier. Our conclusion from the phase I study[14] and extensions of that study (R. Yarchoan and S.B., unpublished data) is that during short courses of administration AZT can bring about partial restoration of immune function and improve certain other clinical abnormalities associated with AIDS. The limiting toxicity observed was bone marrow suppression, predominantly in the form of anaemia and neutropenia. In some patients with HTLV-III associated dementia, AZT brought about quite prompt and dramatic neurologic improvement; however, the durability of the improvement is still not known[61].

AZT was recently tested in a randomized study of patients with fulminant AIDS and poor prognostic AIDS-related complex under double-blind, placebo-controlled conditions at several centres. The study, which was begun in February 1986, had about 140 patients in each group. By September 1986 there were 19 deaths in the placebo group and only one in the AZT group ($P < 0.001$ by Cox's regression model). AZT-treated patients showed other evidence of clinical and immunological improvements. At that point, an independent data-monitoring board recommended that the placebo group begin to receive the drug. The study is still under way (with both groups now receiving drug) and the long-term safety and efficacy are now being assessed. Nevertheless, the results at hand warrant the initiation of appropriate controlled studies to see if therapy with AZT can block the onset of AIDS in individuals who have recently been infected by HTLV-III but who are otherwise free of symptoms.

Thus, AZT, a drug chosen on the basis of its selective *in vitro* antiviral effect against HTLV-III, has been shown to confer a clinical benefit in patients with advanced disease. In this respect, it represents a first step in developing practical chemotherapy against pathogenic human retroviruses. Although the clinical results might be important in their own right, they are also important because they appear to validate the idea that therapy for established retroviral infections is a feasible goal.

Conclusion

The genome of the virus that causes AIDS contains the standard replicative genes found in other retroviruses; however, it contains several additional genes. The product(s) of each gene represents a potential target in developing experimental therapies for diseases caused by this virus, and there are several steps related to the viral lifecycle which may be relevant for future therapeutic strategies. Curative therapy for diseases caused by pathogenic retroviruses will probably not be possible until the molecular biology of the virus and the structural chemistry of the key viral products are defined through further basic research, but it is not necessary to await new data before implementing some strategies that we already know have clinical applications in patients with advanced disease.

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Mass distribution in spiral galaxies

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The masses of a carefully selected sample of primary disk galaxies orbited by one or more satellite systems have been calculated both from the rotation of the disks and from the kinematics of the satellites. The ratio of these two mass estimates (χ_{obs}) is sensitive to the presence of any extended halo and for satellite-primary pairs the mass distribution is significantly different from that of binary galaxies. Our data cannot be described by the heavy halo model with circular orbits, though extended haloes are a possibility if the satellite orbits are highly eccentric.

PERHAPS the most important questions of contemporary, extragalactic astronomy concern the dark haloes that may surround disk galaxies. A popular view is that a typical halo is considerably more massive than the underlying disk, and that it extends out to several Holmberg radii[1]. However, we believe that the observational basis for this surmise is not compelling. Massive haloes are not a necessary ingredient to produce flat rotation curves in disks. Van der Kruit[2] has discussed alternative methods and Hunter *et al.*[3] have considered in detail the effect of truncating a disk. Also, massive haloes are not required to maintain stable disks[4]. While it seems likely that much unseen matter must reside in rich clusters of galaxies[5], we believe the conclusion for small groups and for binary galaxies is less certain.

For small groups and binary galaxies, the observations provide radial velocities and angular separations of suspected members, which are assumed to be gravitationally bound. However, since the projection factors and orbital elements are unknown, the observations must be interpreted in a statistical fashion. Moreover, in the case of binary galaxies, the mass ratio of the members (and, hence, the location of the barycentre) usually is uncertain. Also, it is not clear that the members can always be treated as point masses. Recently, van Moorsel[6] observed members of a carefully selected sample of binary, disk galaxies in H I, with a precision of $\leq 10\ \mathrm{km\ s^{-1}}$. Since his observations provided a 21-cm rotation curve for each galaxy, the individual interior rotation masses could be calculated. Then, assuming that the orbital inclinations were distributed randomly, van Moorsel projected a large number of model pairs upon the plane of the sky, incorporating selection effects and a variety of assumptions about the orbital eccentricities, separations and the amount of dark matter associated with each disk. He concluded that his observations were best fit by models in which the mass of each halo is ~5 times that of its underlying disk, a result which he found to be insensitive to the orbital eccentricities and separations. We have carried out a similar analysis of the Magellanic Cloud-like satellites surrounding several large, disk galaxies and find less certain results.

Method

In observations[7] of H I emission from the barred spiral galaxy NGC3992 the disk of the galaxy was well resolved at a high signal-to-noise ratio, and in addition, emission was detected from three small satellite systems. This makes it possible to determine the mass of NGC3992 by analysis of the kinematics of the disk (the rotation mass) as well as from an analysis of the motion of the satellites (the orbital mass). In our observations, the orbital mass measures the distribution of matter in a volume whose radius is ~3.5 times that of the disk. Therefore, if a galaxy has a significant and extended halo, there should be a pronounced difference between the orbital and the rotation masses. In essence, the satellites are assumed to be test particles orbiting a central mass. In principle this method is superior to conventional studies of binary galaxies, because our satellites typically have ~2.5% of the mass of the primary and in all cases <8%. (This has been determined from the H I, global profiles of the satellites.) Hence, interaction effects should be negligible. We are examining the gravitational potential field of an undisturbed primary system, and in some cases we are exploring this potential simultaneously with several independent probes. The method is superior to optical mass estimation techniques, owing to the precision of the radio velocity measurements ($\varepsilon \leq 10\ \mathrm{km\ s^{-1}}$). Unlike the studies of Peterson[8], or of Helou *et al.*[9], the rotation law is observed directly. Following the suggestion of Lequeux[10], the 'rotation mass' can be estimated from a keplerian model ($M = rv^2/G$). By definition, this estimate includes the gravitational effects of all the mass (disk and halo) within the radius of the most distant observed point in the H I disk. This procedure may overestimate M, for were the mass confined to a thin disk, the total mass would be in the range 0.6–0.76 of the spherical distribution[6,10]. The keplerian mass was used in our calculations.

In our analysis, we have adopted a method described by van Moorsel[6], in which each group is treated as a multiple binary system. Then, we may write the orbital mass as,

$$M \cdot \chi(\nu, i, \omega, e) = \frac{rV_r^2}{G} \tag{1}$$

with

$$\chi = \frac{\sin^2 i}{1 + e\cos\nu}[\cos(\nu + \omega) + e\cos\omega]^2 \times [1 - \sin^2(\nu + \omega)\sin^2 i]^{1/2} \tag{2}$$

where r and V_r are the projected radial separation and radial velocity difference. The function χ depends upon the true anomaly, ν, the angle between the line of nodes and the major axis of the orbit, ω, the inclination of the orbital plane, i, and e, the eccentricity of the orbit. As a measure of the amount of dark matter we have

$$\chi_{obs} = \frac{rV_r^2}{G(m_1 + m_2)} \tag{3}$$

where m_1 and m_2 are the disk masses of the binary galaxies. In our case $m_2/m_1 \ll 1$ and, hence, m_2 can be ignored and the subscripts dropped. χ_{obs} is related to χ by

$$\chi_{obs} = \frac{M}{m}\chi \tag{4}$$

From a Monte-Carlo analysis, we have verified van Moorsel's conclusion that the probability distribution, $P(\chi)$, is peaked sharply for small values of χ. In other words, there is a strong tendency to underestimate the orbital mass (by almost an order of magnitude), because small projected radii and velocities are

Table 1 Primary groups

System	Galaxy type*	V^{+}_{sys} (km s^{-1})	Distance to primary galaxy (Mpc)	Inclination angle of primary disk (deg.)	R_{max} (kpc)	$V(R_{max})$ (km s^{-1})	$m(V)$‡ ($\times 10^{10} M_{\odot}$)	mag	Notes
NGC224	SAS3 SbI-II	−201	0.7	78	28.0	230	34.4	4.3	Rotation curve from CO measurements[22], optical velocities from ref. 23.
NGC1023	SBO- SBO1(5)	610	7.5	80	9.5	251	13.9	10.5	Rotation curve from ref. 24, H I measurements from ref. 13.
NGC1961	SXT5 Sb(rs)IIpec	3935	41.3	50	24.0	400	75.0	12.2	H I measurements and rotation curve from ref. 14; mass calculations from ref. 12.
NGC3359	SBT5 SBc(s)I.8pec	1009	11.0	51	20.8	140	9.5	11.0	H I measurements from ref. 25.
NGC3992	SBT4 SBb(rs)I	1046	14.2	53	19.0	240	25.4	10.7	H I measurements and calculations from ref. 18.
NGC3992	SBT4 SBb(rs)I	1046	14.2	53	19.0	240	25.4	10.7	H I measurements and calculations from ref. 18.
NGC4303	SXT4 Sc(s)I.2	1556	12.9	29	12.2	160	7.3	10.9	H I measurements from refs 27 and L.K.E., distance of group from ref. 28.
NGC4731	SBS6 SBc(s)III	1490	10.5	54	13.0	150	6.8	6.0	H I measurements and calculations from ref. 18
NGC5084	L(−2) SO1(8)	1721	15.0	>86	34.0	328	85.0	—	H I measurements and calculations from ref. 17.

* Morphological type of the primary galaxy, from refs 20, 21.

† The measured heliocentric systemic velocity of the primary galaxy.

‡ Keplerian disk mass calculation using $m = V(R_{max})^2 R_{max}/G$.

§ The apparent blue magnitude from the Uppsala catalogue[11].

highly favoured. On the assumption that the rotation law of the primary reflects all of its mass, and that the system is bound, $P(\chi)$ should fall to zero when $\chi = (1+e) \leqslant 2$.

Selection criteria

Thus, our approach to the general problem of the mass distribution in spiral galaxies is to observe large spiral galaxies (primaries) surrounded by one or more relatively massless satellites. These small groups are found by systematically searching the Uppsala General Catalog[11]. The primaries must be large enough to be resolved at λ 21 cm by the Very Large Array (VLA) telescope and the primaries and satellites have to be rich enough in atomic hydrogen that their kinematic properties can be established with high precision. Furthermore, we desire (primary+satellite) systems which are compact enough in their distribution that they can be observed in one field (diameter ≈ 30 arc min) by the VLA. Finally, by restricting the velocity differences within the groups, we hope to have eliminated most of the problems associated with contamination by field galaxies. The dominance of the primary galaxy is ensured by requiring that it be two magnitudes brighter than, or 1-2 arc min larger than, any other member of the group. We have rejected satellites with velocity of differences $\geqslant 600$ km s^{-1}, a limit imposed by our receivers. (Van Moorsel found that such systems mostly were non-physical pairs.) Six groups surrounding the primary galaxies were indicated by our selection criteria: NGC1961, NGC3992, NGC4111, NGC4258, NGC4303 and NGC3893. The first two were considered earlier, for other reasons, by Gottesman *et al.*[12] and by Gottesman and Hunter[7].

Results

We have augmented our results from the small groups by adding galaxies that we have observed previously, which possess only single satellites (NGC3359, NGC4731 and NGC5084). Also, we have taken information from the literature on M31 and NGC1023. The data for the primaries and their disk masses are given in Table 1, and the data for the satellites and the calculations of χ_{obs} are shown in Table 2. In Fig. 1 we have plotted the distribution of χ_{obs}. The diagram displays 20 primary-satellite pairs (which is four more than the number of binary systems of van Moorsel); of these only four have $\chi_{obs} > 2.0$. By comparison, more than half of van Moorsel's binaries showed $\chi_{obs} > 2.0$. Therefore, he concluded that his disk systems contain haloes with masses typically five times their rotation masses. This is not the conclusion we would draw from Fig. 1. With the exception of the four groups we discuss below, the χ_{obs} distribution can be described by a population of bound satellites. We show in Fig. 1 model distributions for various orbital eccentricities. The theoretical and observed distribution were compared using a Kolmogorov–Smirnov test. The distributions are the same at better than the 5% confidence level and, therefore, no masses other than the rotation masses of the primaries are needed to bind these systems. As we are considering relatively massless satellites, we believe our technique is more risk-free than that of van Moorsel. Both van Moorsel and we are examining the consequences of point source, two-body dynamics, a situation more closely realized in our context than in his. Nonetheless, without further examination, the explanation for the apparent disagreement between the two results is not obvious.

NGC1023 group. Sancisi *et al.*[13] believe that this is an SO that has recently undergone a tidal encounter. The mass of the system from the 'tidal rings' is 6.5×10^{11} $M_{\odot}$ ($R = 45$ kpc) compared to 1.8×10^{11} $M_{\odot}$ within the optical dimensions ($R = 12.6$ kpc). The two dwarfs listed in Table 2 are well isolated from any tidal phenomena, and, as no inclination corrections have been applied to the disk masses, our χ_{obs} values are upper limits. Thus, bearing in mind the unusual nature of this galaxy, it appears to have a more extended mass distribution than the optical results would indicate.

NGC1961 group. This group was studied by Shostak *et al.*[14] using the Westerbork synthesis telescope. Gottesman *et al.*[12] used the satellites to measure the mass of the primary. The satellite UGC3349 indicates a χ_{obs} of ~6, where we have used the smaller of the two mass estimates from Shostak *et al.*[14]. This may indicate a significant halo for the primary. However, we caution that NGC1961 is a peculiar system. The group appears to be embedded in an intergalactic medium which, it is suggested, is stripping the H I from NGC1961. The presence of this

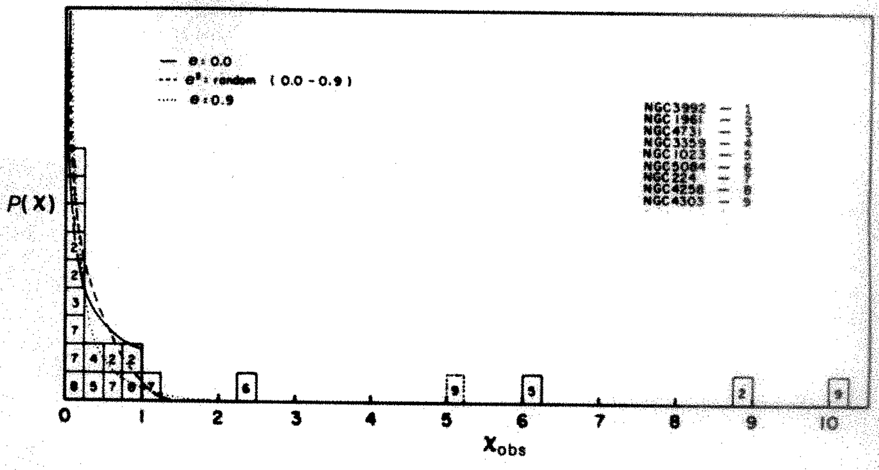

Fig. 1 The theoretical distribution of χ compared with the observed distribution (χ_{obs}). Each rectangle represents one primary-satellite pair for which the primary is identified. The dotted value for NGC4303-UGC7439 is taken from van Gorkom's data. Three model distributions are shown with different orbital eccentricities. The distributions are normalized to the same area.

extended, low density component may complicate the dynamical analysis.

NGC4303 group. This group is made up of three satellites in the field of the VLA telescope. Our observations, which are in progress, show a strong detection for UGC7439 and a possible detection of UGC7404 which we have not listed in Table 2, as it is in conflict with a measured optical value. Regardless, the satellite velocities are large and, consequently, the orbital mass is large too. Our observed maximum velocity in the disk is very small, ~79 km s^{-1} at a radius of ~11 kpc. Thus, a substantial correction for the inclination may have to be made. J. van Gorkom (personal communication) has allowed us to use her data, with better angular and velocity resolution than ours. We find that the inclination could be as small as 17°, less than our data indicate. This inclination is within the range of values quoted by Huchtmeier *et al.*[15] of $14° < i < 48°$. Also, Rubin *et al.*[16] predict a maximum rotation velocity of 209 km s^{-1} for a typical Sc of luminosity class I.1. This would imply an inclination of 18° from van Gorkom's data. Table 2 lists the values from our data, but even if the maximum rotation velocity is 225 km s^{-1} (van Gorkom's data) $\chi_{obs} = 5.1$ for UGC7439. This would imply the presence of a substantial dark halo. However, if the inclination is closer to 48° rather than 14°, the secondaries may not be bound. Also it is possible that the environment of the Virgo cluster adds to the dynamical complexities of this small group.

NGC5084 group. The observations were made by Gottesman and Hawarden[17]. The primary is a peculiar, massive SO$^+$ object with an anomalous gas/dust annulus. The calculated χ_{obs} value

Table 2 Satellites

Group satellite	Heliocentric velocity (km s^{-1})	Measurement method	del V (km s^{-1})*	Separation of satellite and galaxy (kpc)	M(del V) ($\times 10^{10} M_\odot$)†	χ_{obs} (M/m)	mag‡
NGC224	−301	H I					4.3
NGC147	−168	opt	142	90.2	42.3	1.23	12.0
NGC185	−208	opt	100	85.6	20.0	0.58	11.0
NGC205	−240	opt	62	7.5	0.7	0.02	9.4
NGC221	−216	opt	84	4.9	0.8	0.02	9.2
NGC1023	610	H I					10.5
North	905	H I	295	34.2	85.9	6.18	—
South	695	H I	85	17.5	3.7	0.26	—
NGC1961	3,935	H I					12.2
A	4,108	H I	173	91	62.6	0.84	—
B	3,895	H I	40	106	3.9	0.05	—
B1	3,800	H I	135	122	52.4	0.70	—
UGC3342	3,927	H I	8	157	0.2	0.00	15.4
UGC3349	4,282	H I	347	240	671.9	8.96	14.4
NGC3359	1,009						11.0
dwarf	962	H I	47	48.5	2.5	0.26	—
NGC3992	1,046	H I					10.7
UGC6923	1,062	H I	16	60.6	0.4	0.02	14.1
UGC6940	1,112	H I	66	35.4	3.6	0.14	16.0
UGC6969	1,115	H I	69	45.3	5.0	0.20	15.5
NGC4258	465						9.6
UGC7356	255	opt	210	18.8	19.3	0.99	17.0
UGC7335	490	H I	25	19.1	0.3	0.02	13.9
NGC 4303	1,556						10.9
UGC7439	1,270	H I	286	39.1	74.4	10.19	14.9
NGC4731	1,490	H I					6.0
RNGC4731a	1,505	H I	15	32.1	0.2	0.03	—
NGC5084	1,721	H I					—
dwarf	2,089	H I	368	65.6	206.5	2.43	—

* The absolute value of the velocity difference between the primary systemic velocity and the satellite velocity; this is the quantity V_r in equation (1). Note that the NGC224 velocities are in the ASR reference frame, not heliocentric.

† The orbital mass of the primary galaxy computed from $M = (\text{del } V)^2 R/G$, with R the separation between the primary and satellite galaxies.

‡ The apparent blue magnitudes of the members, from the Uppsala catalogue[11].

of ~2.4 is at the limit of significance considering the uncertainty in the extreme inclination of the disk, and of the systemic velocity of the satellite. (The global H I velocity profile of the satellite is not symmetrical.)

NGC3992 group. This group has been discussed extensively by Gottesman and Hunter[7] and by Gottesman *et al.*[18]. The disk mass we have used comes from a keplerian calculation at $R_{\max}$. Thus, the mass differs slightly from that given by Gottesman *et al.* None of the observations reveal the presence of a massive halo. Dynamical modelling of NGC3992 by Hunter *et al.*[19] leads to similar conclusions. We believe that the three independent analysis of the data indicate most strongly that this system does not have a significant halo. Indeed, it may be the best example of such a spiral galaxy.

Thus, from our observations, at most four groups have χ_{obs} values >2. Hence, the primary galaxies in these systems may contain massive haloes. In contrast NGC3992 almost certainly does not have a massive halo. What requires further interpretation is the observed distribution of χ.

Discussion

Van Moorsel found that he could bind his pairs if he assumed a model in which the flat rotation curves of both galaxies were extended until the mass distributions touched. We have considered models of this type. We assume the satellites to be massless points moving in the potential of the halo of the primary. The mass of the primary is obtained by extrapolating a flat rotation curve out to the distance of each satellite. ($M \propto R$; halo density $\propto R^{-2}$.)

Following van Moorsel, we write,

$$\chi' = \frac{R}{r}\chi = \left[\frac{V_r}{V(R_{\max})}\right]^2 \tag{5}$$

where R is the true separation of the satellite–primary pair, and r is the observed separation. Assuming the data are unbiased, the hypothesis that the model correctly describes the observations can be rejected. That is, we conclude from a Kolmogorov–Smirnov test that van Moorsel's simple halo model, involving circular orbits, will not reproduce our observations. There are too many small values of χ. However, this model may not be appropriate if violent collapse of the haloes of the primaries caused the satellites to have radial orbits. We have not explored this situation exactly, for if the mass of the primary is proportional to r then the gravitational force on a satellite is proportional to r^{-1}, and the orbits must be recalculated. As we are sampling the satellites at different locations in their orbits we have approximated the real situation by a simple scaling of the (point) orbital mass appearing in χ. The scaling factor is an adopted value of R/r. This ratio ranges from 0.2 to 12, for our data, with an average value of 3.5.

The shortcoming of this model is that it greatly overcalculates the forces and velocities near pericentre but not at apocentre. Thus, χ values predicted by the model are too large at small separations. However, more detailed modelling is warranted because we find that χ does not correlate with projected separation. We conclude that with the exception of the four unusual systems we have discussed, the χ_{obs} distribution can be modelled successfully by galaxies whose masses equal the keplerian mass defined by the last observed point in the disk. No massive haloes are required to bind the satellites. In addition, our data canr[illegible] fit van Moorsel's halo model with circular orbits, and as R[illegible] increases, satisfactory models only can be constructed by assuming increasingly eccentric orbits for the satellites (for $R/r = 2.5$, $e \geqslant 0.6$; for $R/r = 3.5$, $e \geqslant 0.9$; for $R/r = 5$, $e > 1$).

While this approach is not completely satisfactory, it does represent an upper limit, for the satellites feel the full effect of the mass of the primary at all locations in their orbits. Thus, our data probably cannot accommodate halo masses in excess of ~3 times their keplerian masses, and such an increase in the masses of the primaries requires the satellite orbits to be very eccentric.

If the model presented here is correct, then NGC3992 is unusual in that it does not have an extended halo. In contrast, NGC1023, NGC1961, NGC4303, and perhaps NGC5084, either must have massive haloes or be unbound systems.

Throughout this discussion we have presumed that our data sample is unbiased. However, at least three of our primaries are strongly barred and our χ_{obs} distribution may not reflect a homogeneous sample of disk galaxies. Also, the χ_{obs} distribution may reflect selection effects in our database. Other, differences between van Moorsel's sample and ours are that he explored a larger volume (relative to a typical disk) than we have, and our groups cover a wider range of separations. Van Moorsel found χ_{obs} to be very insensitive to the applied selection criteria. However, as we chose galaxy pairs with larger magnitude differences, our data may be biased differently. Following van Moorsel's precepts, we have created a synthetic UGC[11] and from this we have modelled our selection effects. Like van Moorsel we find a pronounced difference between the biased and unbiased distribution of galaxy separations. However, again like van Moorsel, we find, that there is no significant difference in the mean values of χ for the two distributions. We can only echo his conclusion; 'the distribution of the parameter χ is v[illegible] insensitive to the applied selection'. Hence, the observed distributions are different.

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Bipolar affective disorders linked to DNA markers on chromosome 11

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An analysis of the segregation of restriction fragment length polymorphisms in an Old Order Amish pedigree has made it possible to localize a dominant gene conferring a strong predisposition to manic depressive disease to the tip of the short arm of chromosome 11.

ESTABLISHING the role of genetic factors in the aetiology of mental illness has represented a formidable challenge. The separation of environmental from intrinsic biological factors and the complexities of psychiatric diagnosis are major obstacles in this endeavour. Nevertheless, evidence of biological and genetic contributions to aetiology make the major affective disorders excellent candidates to address this issue.

Major affective disorders are a group of illnesses manifested by disturbance in mood, and in physiological, cognitive and endocrine functions[1]. The primary forms are unipolar major depression and bipolar disorder, distinguished by the occurrence in the latter of manic episodes, typified by an elevated, expansive or irritable mood. Symptoms of mania can include physical restlessness, increased talkativeness, racing thoughts, inflated self-esteem extending to delusions of grandeur, decreased need for sleep and distractability. Manic episodes usually lead to impairment in both social and occupational function because of excessive behaviour patterns. Hypomania is less severe and has fewer symptoms. When mania or hypomania occurs in association with depressive episodes, the diagnosis is bipolar I (BP I) or bipolar II (BP II) disorder, respectively.

Depressive episodes are characterized by a mood of hopelessness. Associated symptoms are appetite and weight changes, sleep disturbance, difficulty in concentrating, decreased energy, loss of interest, and feelings of worthlessness or guilt, extending to thoughts of death or suicide. Major and minor depression are distinguished by the number and severity of these symptoms.

Bipolar disorder (manic-depressive illness) has an estimated lifetime prevalence of ~1% in North America and western Europe[2]. Onset is usually between 15 and 35 years of age. Major depression has a much higher estimated prevalence with a wider range in age of onset[2]. Both manic and depressive episodes can persist for periods of a week to several months and may require hospitalization.

The response of manic patients to lithium salts[3] and of depressed patients to the tricyclic antidepresants, monoamine oxidase inhibitors and related medications[4,5] indicates that neuronal systems affected by these agents are important in these illnesses. The biological basis of manic and depressive states also is evidenced by cycling of mood unrelated to specific situational factors and by altered patterns in energy, sleep and appetite. Twin studies, adoption studies and family studies demonstrate that genetic factors can play an important role in the aetiology of affective disorders[6-10]. However, establishing the mode of transmission and identification of a major genetic locus has been problematic. To evaluate the genetic contribution to major affective disorders, a study of the genetically isolated Old Order Amish (OOA) population in southeastern Pennsylvania (the Amish Study[11]) was initiated in 1976.

Amish study

The Amish study has presented a unique opportunity to examine hypotheses of genetic transmission. Detailed genealogical records document that all the 12,000 members of the OOA population are descended from 30 progenitors who emigrated from Europe in the early eighteenth century[12]. Over the past 25 years this closed community has been studied extensively by one of us (J.A.E.). Large family size, clearly established paternity and geographic concentration made it excellent for epidemiological and pedigree studies. Social and religious prohibitions of alcohol and drug use permitted more accurate ascertainment and diagnosis of affective disorders. Prevalence of affective disorders among the OOA was found to be similar to the general North American population[13]. The characteristics of the major forms of affective disorder, including basic symptoms, age of onset, course of illness and response to pharmacological treatment, were indistinguishable for Amish and non-Amish patients.

To date, the study has focused on families of bipolar patients. Segregation analyses of these family data indicate that an autosomal dominant mode of transmission is most consistent with the patterns of illness observed in the kindreds. Results suggest that the allele predisposing individuals to bipolar affective disorders has a frequency of 0.021 ± 0.002 in the population[12]. Penetrances were estimated using age-specific estimates of the population prevalence for the illness. The susceptible genotypes (AA and Aa) were estimated to have a maximum penetrance of 0.63 at age 30 and above. The 'normal' genotype (aa) was estimated to have penetrance 0.0001. These results provided a strong impetus to genetic linkage studies.

Initial linkage studies, including information from a number of polymorphic serum proteins and blood-group antigens did not give evidence for linkage to a major locus for affective disorders[14]. Recombinant DNA techniques provide a vehicle for dramatically expanding the number of markers available for genetic linkage studies through the identification of restriction fragment length polymorphisms (RFLPs)[15]. To implement this strategy in the Amish study, blood samples were collected from a multigenerational pedigree, highly informative for bipolar illness. Permanent lymphoid cell lines were established from these blood samples at the Coriell Institute for Medical Research[16]. DNA derived from 51 of these cell lines was used in an initial series of RFLP studies. Preliminary evidence was obtained suggesting possible linkage between a major locus for bipolar illness and two markers on chromosome 11, insulin (*INS*) and the cellular oncogene Ha-*ras*-1 (*HRAS1*)[17,18]. To extend these studies, DNA samples were prepared from permanent cell lines established from 30 additional members of

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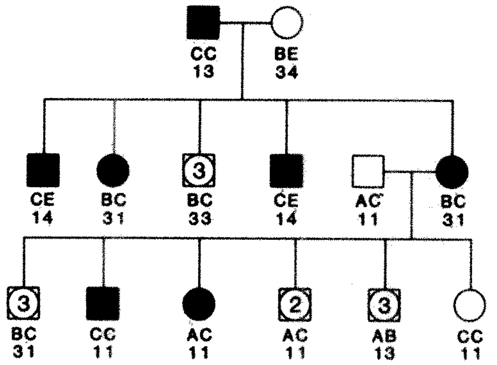

Fig. 1 *INS* and *HRAS1* typings on selected members of Pedigree 110. Both the *INS* and *HRAS1* loci have multiple alleles as a result of an insertion/deletion sequence in the immediate vicinity of the coding region[24,25]. Four different alleles at the *INS* locus were observed when genomic DNA of the individuals shown in this figure was digested with the enzyme *Pvu*II. The alleles are: A, ~760 base pairs (bp); B, ~800 bp; C, ~830 bp; D, ~2,300 bp. Alleles of *HRAS1* are as follows: 1, 5.2 kilobases (kb); 2, 5.7 kb; 3, 6.2 kb; 4, 6.8 kb; on *Sac*I-digested DNA. Filled symbols, individuals with affective disorder; open symbols, individuals without affective disorder to date. Squares, males; circles, females; superimposed square and circle, other siblings (number given in centre). Note that alterations in sex designation and reductions in sibship size have been made to preserve anonymity of individuals at risk of affective disorder.

Methods. DNA from each individual was digested by either *Sac*I (*HRAS1*) or *Pvu*II (*INS*), electrophoresed on 0.7% Tris-acetate agarose gel (*HRAS1*) or 1.2% Tris-borate agarose gel (*INS*), tranferred to Xetabind (nylon-based membrane, AMF-Cuno) by a method[44] modified from Southern. Filters were pretreated in 0.5% SDS, 0.1 × SSC at 60 °C for 1 h and prehybridized overnight in 50% formamide, 6 × SSC, 50 mM sodium phosphate pH 6.5, 5 × Denhardt's solution, 100 μg ml^{-1} sonicated, denatured salmon sperm DNA and 0.5% SDS. Hybridization was carried out in the above solution but with only 25 mM sodium phosphate and 1 × Denhardt's solution) for two days, using as probes the plasmid phins310 for *INS* and pEJ6.6 for *HRAS1*. Incubation temperature was 50 °C for *INS* and 42 °C for *HRAS1*. Filters were washed once at room temperature in 2 × SSPE, 0.1% SDS, twice at 55 °C in 2 × SSPE, 0.1% SDS and once at 55 °C in 0.1 × SSPE, 0.1% SDS. SSC (1 ×) is 150 mM NaCl, 15 mM sodium citrate.

this pedigree[16]. We now report compelling evidence for tight linkage between two DNA sequences located on chromosome 11 and a locus conferring a strong predisposition to bipolar affective disorders.

Case ascertainment and diagnoses

Methods for case ascertainment included both a community-wide epidemiological survey among the Amish and a search of all Amish medical records at local psychiatric hospitals. Multiple interviews of patients and family members using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version[19] (SADS-L) and abstracts of hospital and clinic records formed the basis for diagnosis. Diagnostic evaluations were made by a psychiatric panel blind to patient identity, biological relationships, previous diagnoses and treatment response. When both interview and medical abstracts were available, they were evaluated separately. The panel adhered strictly to research diagnostic criteria[20] which define mood, symptoms, duration and severity. High diagnostic reliability was demonstrated[21].

Thirty-two bipolar families were identified from a larger sample of psychiatrically ill patients. The linkage studies reported here are based on one large composite family (pedigree 110) which encompasses three of the 32 bipolar families originally ascertained. We focus on 81 members from pedigree 110 including 19 affected and 62 psychiatrically well individuals.

Of the 19 subjects with major affective disorders, 14 had some form of bipolar affective illness. Based on research diagnostic criteria, 11 were diagnosed as bipolar I, one was diagnosed as bipolar II, one had a schizoaffective disorder, manic-depressive type and one had atypical psychosis with prominent affective features. Five members of pedigree 110 suffered from unipolar major depression. An additional five individuals were diagnosed with minor psychiatric conditions but were considered unaffected in the linkage analyses. Systematic screening methods were used to update changes in psychiatric status on a yearly basis for pedigree 110. Consequently, the diagnoses used [illegible] these analyses do not correspond completely to those origin[illegible] published for this family[16].

Genetic markers and linkage analysis

DNA samples from members of pedigree 110 were typed (blind to diagnosis) for segregation of a number of loci located on chromosome 11. The results for two loci close to the distal end of the short arm of the chromosome, *INS* and *HRAS1*, are presented here. Both these loci, located ~3 map units apart[22,23] exhibit high frequency RFLPs due to the insertion or deletion of a short repeated DNA sequence in the non-coding region adjacent to the gene[24,25]. The multiple alleles that are a consequence of these mutations were generally adequate to provide segregation information in most meioses. Because several individuals were homozygous for the insertion/deletion polymorphism, a *Taq*I site polymorphism outside the *INS* gene[26] was also typed. Haplotypes were then established for the insertion/deletion and the *Taq*I polymorphism. The resulting haplotypes were used in linkage analyses.

The segregation of the *INS* and *HRAS1* loci in a segment of pedigree 110 is shown in Fig. 1. In the two oldest generations, allele 1 of *HRAS1* and the C allele of *INS* cosegregate with the occurrence of bipolar affective disorder. All members of these two generations are past the upper end of the age-of-onset spectrum for bipolar disorders. In the third generation all affected individuals have also inherited the 1 allele at *HRAS1* and the C allele at *INS* from an affected parent. All well individuals who received this combination of alleles from an affected parent were still within the age of risk for developing the illness.

To determine the statistical significance of cosegregation of these markers with a proposed gene for predisposition to bipolar affective disorder, linkage analyses were performed using the computer program LIPED[27], modified to allow for age-dependent penetrance[28]. The accuracy of these analyses is dependent upon correct assessment of clinical status as well as the specification of correct values for penetrances and gene frequen-

Table 1 Estimates of gene frequency and age-specific penetrance for affective disorder from the Amish study

	Autosomal dominant model			
	Total sample		Pedigree 110	
	$q = 0.021 \pm 0.002$		$q = 0.015 \pm 0.005$	
Age	f_{A-}	f_{aa}	f_{A-}	f_{aa}
15–19	0.10	0.0000	0.18	0.0001
20–24	0.27	0.0001	0.42	0.001
25–29	0.56	0.0001	0.73	0.001
30+	0.63	0.0001	0.85	0.001

Quantities: q, frequency of the susceptibility allele A; f_{A-}, penetra[illegible] for genotypes AA or Aa; f_{aa}, penetrance for genotype aa. Segregation analyses were performed using the computer program POINTER[42]. The total sample included relatives of all 32 BP I probands ascertained from the OOA population. All relatives younger than 14 were excluded from the analyses. For these analyses, relatives with diagnoses of BP I, schizoaffective, BP II or major depressive disorder were included as affected. All other relatives were considered unaffected. Population prevalences incorporated were obtained from the entire OOA community. A wide range of genetic hypotheses were examined in an hierarchical fashion. Likelihood ratio tests were employed to determine statistical significance[13].

Table 2 The lod scores for pairwise linkage analyses between *HRAS1* and a major affective disorders locus for four sets of penetrance values

Maximum penetrance	Recombination frequency (θ)						
	0.000	0.001	0.010	0.030	0.050	0.070	0.100
0.55	3.070	3.068	3.041	2.971	2.888	2.796	2.643
0.63	3.340	3.337	3.310	3.239	3.155	3.059	2.898
0.85	4.083	4.080	4.061	3.997	3.912	3.810	3.631
0.95	4.322	4.321	4.310	4.258	4.178	4.077	3.894

Pairwise linkage analyses were performed with the computer program LIPED[25] modified to include age-dependent penetrance. Analyses were performed assuming a dominant mode of transmission. The estimate of trait allele frequency was fixed at 0.021. Penetrance varied linearly from 0.0 at age 15 to the maximum at age 35 and beyond. Allele frequencies were estimated from a large reference sample as follows: allele 1, 0.3; allele 2, 0.3; allele 3, 0.1 and allele 4, 0.3. A wide range of penetrance estimates for genotypes *AA* and *Aa* were examined. The penetrance of *aa* in all analyses was fixed at 0.001. The values of penetrance used for *AA* and *Aa* included those obtained from segregation analysis of the total sample of 32 BP families and from pedigree 110 alone. In addition, penetrance estimates obtained from the inclusion of the standard error of the parameter estimates from segregation analyses were incorporated. The recombination fraction (θ) varied from 0.5 to 0.10 in increments of 0.05, and from 0.10 to 0 in the increments shown. A lod score represents the logarithm to the base 10 of the likelihood (L) ratio $L(\theta)/L(0.5)$. A lod score of 3.0 represents odds of ~1,000 to 1 that the observations are the result of linkage at the specified recombination frequency rather than independent segregation.

cies[29]. To evaluate the sensitivity of linkage results to alternative values for these parameters, analyses were performed over a wide range of possible values. This range (0.55–0.95) included values within one standard error of the maximum penetrance estimates obtained from segregation analyses of the entire OOA sample as well as from pedigree 110 alone. Maximum penetrance values from the entire OOA sample increase to a value of 0.63 at age 30 or greater (Table 1). The values obtained for pedigree 110 when analysed separately from the other OOA families increased to a maximum penetrance of 0.85 for age 30 or greater (Table 1). The estimate of the trait allele frequency obtained from this family was 0.015.

Evaluation of the likelihood of linkage between *HRAS1* and the proposed affective disorder locus (as lod scores: see Table 2 for definition) provided strong support for linkage between the two loci (Table 2). A lod score of 3.340 at $\theta = 0$ for linkage between the proposed affective disorders locus and *HRAS1* was obtained at a maximum penetrance value of 0.63 based on segregation analyses of the total sample of 32 bipolar families. At this value of penetrance, the lod score continues to be above 3.0 for values of recombination frequency up to 0.07. Given these assumptions, the hypothesis of linkage between the two loci is favoured over the null hypothesis of non-linkage by odds of better than 1,000 to 1.

The lod score was 4.083 at $\theta = 0$ when the maximum penetrance value, 0.85, from segregation analyses of pedigree 110, was used. The lod score remained greater than 3.0 for all values of recombination frequency up to 0.25. If this estimate of penetrance is most accurate for pedigree 110, then odds favouring linkage as the explanation for the data reported here are >10,000 to 1 over the null hypothesis of non-linkage and chance. Odds favouring linkage at $\theta = 0$ remain high for all values of penetrance tested between 0.55 and 0.95 (data not shown). We conclude that linkage analysis supports the location of the proposed affective disorders locus in the region of chromosome 11 which includes the *HRAS1* gene.

To test this conclusion, linkage analyses were carried out between the *INS* locus, located ~3 map units from the *HRAS1* locus, and the affective disorders locus, using the same wide range of penetrance values included in the *HRAS1* analyses. The lod scores obtained provide support for a localization of an affective disorders gene to the short arm of chromosome 11. The lod score was 1.75 at $\theta = 0.03$ (Table 3) using population based estimates of penetrance. Using penetrance values from pedigree 110 yielded a lod score of 2.63 at $\theta = 0.0$. Several factors could account for the somewhat lower values for *INS* compared to *HRAS1*. The most significant is that the affective disorder gene is probably closer to the *HRAS1* locus.

To assess the impact of variation in allele frequencies on the robustness of the findings, analyses were performed varying these estimates. For both *INS* and *HRAS1*, the allele frequencies used in the original analyses were compared to an arbitrary assumption of equal allele frequencies for all marker alleles. The lod scores increased slightly for *HRAS1* and decreased slightly for *INS* under these arbitrary assumptions (D.L.P., unpublished). In addition, analyses were performed incorporating alternative values for allele frequency of the affective-disorder locus. Allele frequencies of 0.010, 0.015, 0.019 and 0.023 were included. In all cases, analyses indicate that the conclusions we reach are not dependent on allele frequencies used as all estimates gave scores with only minor numerical differences. We conclude that the data provide strong support for linkage between the affective disorders locus and the *HRAS1* and *INS* loci.

Multipoint linkage analysis

To evaluate more accurately the relative position of the affective disorders gene and the *INS* and *HRAS1* loci, multipoint linkage analyses were performed using the computer program LINKMAP[30]. Multipoint analyses included information already known about linkage of marker loci by specifying the distances between them and then examining the likelihood of an unmapped locus being at various positions relative to the specified positions of the markers. Three-point analyses were performed to test for linkage of the proposed affective disorder locus to the *INS–HRAS1* region. Data on the relative map positions of *INS* and *HRAS1* were derived through analysis of segregation of these markers in the Venezuela Reference Pedigree and this OOA pedigree[22] (D.S.G. *et al.*, in preparation). Results strengthen and are consistent with the pairwise findings (Fig. 2). The lod score exceeds 3.0 at all map positions within 30 cM of the *INS–HRAS1* region. The slight reduction in the lod score at *INS* (lod = 4.083) implies that the gene for bipolar affective disorder is less likely to be located exactly at the position of this marker. The lod score peaks (at 4.904) at *HRAS1*. These results indicate that the affective disorder gene is most likely to be tightly linked to the *HRAS1* locus on the short arm of chromosome 11.

Implications

It has been suggested that the complex nature of common disorders represents a serious barrier to the application of genetic linkage strategies[31]. For the first time the application of a generalized strategy for scanning the human genome has provided convincing evidence for the localization of a gene that

Table 3 The lod scores for pairwise linkage analyses between *INS* and a major affective disorders locus for two sets of penetrance estimates

Maximum penetrance	Recombination frequency (θ)						
	0.000	0.001	0.010	0.030	0.050	0.070	0.100
0.63	1.671	1.677	1.714	1.752	1.754	1.733	1.674
0.85	2.634	2.632	2.615	2.566	2.503	2.431	2.308

Analyses were performed as in Table 2 except that the following allele frequencies (derived from pedigree 110) for *INS* were used: allele 1 (haplotype A), 0.40; allele 2 (haplotype B), 0.15; allele 3 (haplotype C), 0.39 and allele 4 (haplotype D), 0.06. Data were relabelled to reduce computation time. Relabelling affects numerical results only by altering their specific dependency on marker allele frequency[43].

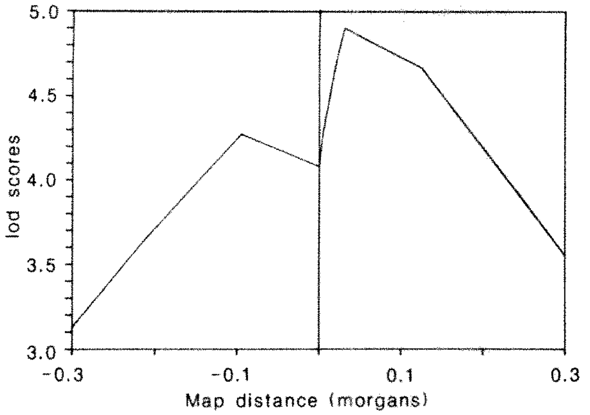

Fig. 2 Multipoint linkage analysis for bipolar affective disorder versus *INS* (at 0.0 morgans) and *HRAS1* (0.031 morgans). **Methods.** Analyses using the computer program LINKMAP[30] were performed incorporating the gene frequency and age-specific penetrance estimates from segregation analyses of pedigree 110: $q = 0.015$; $f_{A-} = 0.18$, $f_{aa} = 0.0001$ for ages 15–19; $f_{A-} = 0.42$, $f_{aa} = 0.001$ for ages 20–24; $f_{A-} = 0.73$, $f_{aa} = 0.001$ for ages 25–29; $f_{A-} = 0.85$, $f_{aa} = 0.001$ for ages 30 and over. Quantities as in Table 1. The trait and marker data used were identical to those used in the pairwise analyses. However, one loop in the pedigree structure had to be broken to allow the program to run. Based on pairwise analyses, the loop was broken in the most conservative way.

is implicated in the aetiology of a common clinical disorder. These findings have broad implications for research in human genetics and psychiatry. The demonstration by a linkage strategy that a simple genetic mechanism can account for the transmission of bipolar affective disorders in pedigree 110 should provide an impetus to analogous research on other common clinial conditions. These illnesses include cancer, cardiovascular disease, diabetes and hypertension as well as other psychiatric disorders.

The results reported raise two major questions in psychiatric genetics. First, to what extent do the genetics of bipolar affective disorders in pedigree 110 reflect the genetics of affective disorders in other families and populations? Second, through what molecular mechanism does a chromosome 11 DNA sequence predispose members of pedigree 110 to bipolar affective disorders?

Genetic and clinical heterogeneity

The other families identified with affective disorders during the Amish study can be of great value in addressing the first of these questions. Data reported here on pedigree 110 show that a number of clinically variant forms of affective disorder (bipolar I, bipolar II, major depressive and schizoaffective) share a common chromosome 11 haplotype. Analysis of direct extensions of pedigree 110 and expansion of linkage studies to other Amish families identified with affective disorders could provide further data on the clinical spectrum associated with the chromosome 11 gene. It will be important to determine whether linkage with chromosome 11 markers is observed in multi-generational Amish families that exhibit forms of major affective disorders other than bipolar illness. If additional families that carry the chromosome 11 affective-disorders locus can be identified, then the existence of genes or environmental factors that modify the expression of penetrance of this locus could be effectively studied.

Although a great deal can be learned through extensions of the Amish study, it is also clear that linkage of chromosome 11 markers for predisposition to affective disorders must be tested in other family studies. A recent report describes cosegregation of thalassaemia and affective disorder in a non-Amish pedigree[32]. As the β-globin locus responsible for β-thalassaemia is located on the short arm of chromosome 11 and is closely linked to the *HRAS1* and *INS* loci[22,23] these findings would indicate that a chromosome 11 affective disorders gene may have been detected in at least one non-Amish family.

Heterogeneity is likely in a syndrome as common as bipolar disorder. A variety of samples of affective disorder families will be required to explore this issue. Given the difficulties of ascertainment of multi-generational families in the population at large, the affected sib pair method[33] is an attractive alternative to complete family studies. A sample of 100–200 affected sib pairs should rapidly determine the extent to which the chromosome 11 locus contributes to the aetiology of affective disorders in the general population.

Heterogeneity would be demonstrated if linkage could be found between a locus for predisposition to affective disorder and genetic markers located in other regions of the genome. Several investigations have suggested possible linkage to markers (colour blindness and the *Xg* locus) on the X chromosome. Because *Xg* and protan/deutan colour blindness loci are located at opposite ends of the X chromosome, the results of these studies conflicted. A number of further studies, using different samples, did not confirm these initial findings[34]. Evidence suggesting linkage between a gene for affective illness and the chromosome 6 locus for human leukocyte antigen system (*HLA*) has been reported[35,36]. Subsequent investigations have been unable to replicate these findings[37,38]. The data from the Amish study of bipolar pedigrees conclusively exclude linkage to *HLA* in this population[14].

Although genetic heterogeneity may explain some apparent contradictions among studies, use of different analytical approaches could also contribute to conflicting outcomes. In previous studies, positive findings were specific to narrow ranges of genetic model parameters. These parameters can significantly influence the outcome of linkage analyses[29]. Thus, these studies do not provide convincing evidence for a possible locus involved in the aetiology of major affective disorders.

The findings reported here are robust over a wide range of genetic model parameters. The lod scores reported for linkage between *HRAS1* and the proposed affective disorder locus are larger than 3.00 for all values of maximum penetrance greater than 0.55 and exceed 4.00 for values of maximum penetrance from 0.85 to 0.95. Furthermore, the application of multipoint linkage analysis provides even stronger support than two-point analyses for the linkage of the proposed affective disorder locus to *INS* and *HRAS1*. Analysis of other RFLPs scattered throughout the genome in the original 51 individuals collected of pedigree 110 yielded no evidence for linkage to those markers[39]. This further strengthens the chromosome 11 findings reported here.

Clinical implications

Although the genetics of affective disorders in the general population is likely to be more complex than in pedigree 110, it is clear that DNA-based diagnostic procedures have a future in psychiatry. The ability to identify individuals at risk by DNA genotype promises significant clinical benefits. Because not all individuals who inherit the gene will manifest the illness, the ability to identify those at risk by direct DNA analysis could permit the design of new studies to examine the interaction of genetic and environmental factors important for the onset of illness. Such prospective studies, although attractive theoretically, raise serious ethical and social considerations. Similar caution must be exercised in the direct use of predictive information in a clinical setting.

The second major issue raised by our findings is the precise identification of the physiological or biochemical mode of action of the gene responsible for bipolar disorders within pedigree 110. The clinical response of patients to therapeutic agents provides a framework for integration of insights from molecular biology once this gene is identified. As a number of expressed DNA sequences have been mapped to the short arm of chromosome 11, several possible candidate DNA sequences for the proposed affective disorders locus have already been identified. For example, the structural gene encoding tyrosine hydroxylase, mapped to the same segment of chromosome 11 as *INS* and *HRAS1*[40], deserves consideration, because this enzyme catalyses an important step in the dopamine synthesis pathway. It has been suggested that defects in dopamine metabolism play an important role in the development of major mental disorders[41].

Elucidation of the mechanism of action of the affective-disorders gene should lead to a better understanding of the aetiology of affective illness. It will be satisfying if insight into the molecular genetics of affective disorders leads to improved treatment for this serious and prevalent psychiatric condition.

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LETTERS TO NATURE

Shocked bipolar outflow from the evolved star OH231.8+4.2

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Low- and intermediate-mass stars lose significant fractions of their mass during the asymptotic-giant-branch evolution, creating massive circumstellar shells. The deeply enshrouded OH infrared stars are presumably at the end of asymptotic-giant-branch evolution. I report here the discovery of shocked bipolar bubbles expanding supersonically at right angles to a dense dust disk around the red-giant OH infrared star OH231.8+4.2. The relative radial velocity between the northern and southern bubble fronts is over $200\ \mathrm{km\ s^{-1}}$, and with a total physical extent of 0.42 pc and a known inclination, this gives a dynamical age of about 1,500 yr. Herbig–Haro objects are observed at the front of the bubbles, and are probably formed as instabilities in the bow shocks, where two collimated flows ram into the ambient medium. This new phenomenon represents a brief phase of late stellar evolution, immediately preceding the formation of a planetary nebula.

OH231.8+4.2 (also known as OH0739−14) is a strong OH infrared source, surrounded by an elongated near-infrared reflection nebula of ~9 arc s[1], which coincides with a faint optical nebulosity extended along the same direction[2]. Optical spectroscopy[3] indicates a spectral type of the hidden star of M9. Monitoring of infrared luminosity[4] and OH-maser intensity[5] shows a regular periodicity with a period of ~650–680 days. Mapping of the 1,667-MHz OH maser emission with high spatial and velocity resolution shows bipolar large-scale velocity gradients along the optical–infrared axis[5,6]. Recently, Cohen *et al.*[7] performed long-slit spectroscopy along the axis of the optical nebulosity, and discovered Herbig–Haro (HH) like high-velocity blue- and redshifted shock-excited regions outside the nebula.

Direct charge-coupled device (CCD) imaging was carried out at the Danish 1.5-m telescope at the European Southern Observatory (ESO), La Silla, with a GEC chip having low read-out noise and a pixel size of 22 μm, corresponding to 0.35 arc min on the sky. Figure 1*a* shows a deep $H\alpha$/[N II] interference filter image of OH231.8+4.2. The immediately striking features are the two bubble-shaped lobes emanating from the central object, two collimated streamers and an obscuring ridge separating the southern and northern parts. With an axial extent of 30 and 20 arc s for the southern and northern bubbles, respectively, these are very large objects; at a distance of 1.2 kpc[5]

Table 1 Infrared observations

Wavelength (μm)	Magnitude	Flux (Jy)
1.25	8.19(0.02)	0.85(0.01)
1.65	6.72(0.01)	2.18(0.03)
2.2	5.56(0.02)	3.88(0.06)
3.8	3.28(0.02)	12.3(0.22)
4.8	2.19(0.04)	20.6(0.68)
12		19.0(1.5)
25		226(18)
60		548(44)
100		292(47)

1.25–4.8 μm: ESO 1-m telescope data.
12–100 μm: IRAS data.

and with an inclination of 47° to the plane of the sky (Tielens *et al.*, in preparation) these correspond to 0.25 pc and 0.17 pc (50,000 and 35,000 AU) respectively. The HH objects recently discovered by long-slit spectroscopy are located at distances from the central source corresponding to the front edges of the bubbles. Figure 1*b* shows a similar exposure through a slightly redder filter, excluding the Hα and [N II] lines. Only reflected light and fore- and background stars are visible, and all traces of the bubble structures have vanished, testifying to their emission-line nature. Another similar exposure through a [S II] filter, shows the basic features of the bubbles, but only at very low light levels. The morphology and evolution of this system is discussed later.

Long-slit spectra have been obtained with the ESO 2.2-m and 3.6-m telescopes, with Boller and Chivens spectrograph and CCD-detector, with the slit placed along the symmetry axis. The spectra confirm the finding of Cohen *et al.*[7] that the [N II] lines are of extreme intensity, and that the northern shock region is blueshifted and the southern redshifted. Based on the five lines Hα, [N II] 6,548 Å, [N II] 6,584 Å, [S II] 6,717 Å and [S II] 6,731 Å the heliocentric radial velocity of the northern shock region was measured to be -42 km s^{-1}, and for the southern region $+180$ km s^{-1}, both with a standard deviation of 27 km s^{-1}. In the local standard of rest this corresponds to -60 km s^{-1} and $+162$ km s^{-1}, respectively. Maser emission has been mapped with high spatial and velocity resolution[6], showing that maser emission is almost symmetrically extended about 10 arc s along the polar axis, with $-16\ \mathrm{km\ s^{-1}} < v_{\mathrm{lsr}} < 81\ \mathrm{km\ s^{-1}}$, and a velocity at the source location of $+20$ km s^{-1}. The radial velocity of the northern shock front relative to the central maser velocity is lower than for the southern front (80 compared with 140 km s^{-1}), which possibly accounts for its less developed state. From the known inclination of the system, one finds space velocities of 110 km s^{-1} (N) and 190 km s^{-1} (S). With dimensions of 0.17 and 0.25 pc for the northern and southern bubbles, respectively, this suggests dynamical ages of ~1,500 and ~1,300 yr, which are identical within the uncertainties.

Near-infrared photometry was carried out with the ESO 1-m telescope, and is listed in Table 1, together with IRAS (infrared astronomical satellite) fluxes. These near-infrared data are slightly brighter than the brightest observations of the extended data set of Feast *et al.*[4], so the present data were taken very near to maximum light. The present observation at JD 2446478 offers a long baseline to the early infrared data, and assuming conservatively that it falls within a half-year window around the true maximum of the lightcurve, and assuming another maximum at JD 2444403 in ref. 4, a period of 692 ± 30 days is suggested. The near-infrared variability is considerable, the luminosity between 1.25 and 2.2 μm for the two data sets in Fig. 2 varies from 34 to 96 $L_\odot$, close to a factor three. The energy distribution of OH231.8+4.2 from the data in Table 1 plus a 400 μm observation from Sopka *et al.*[8] is shown in Fig. 2. The luminosity of the data in Table 1 between 1.25 and 4.8 μm is about 420 $L_\odot$, and the data in Fig. 2 give a luminosity between

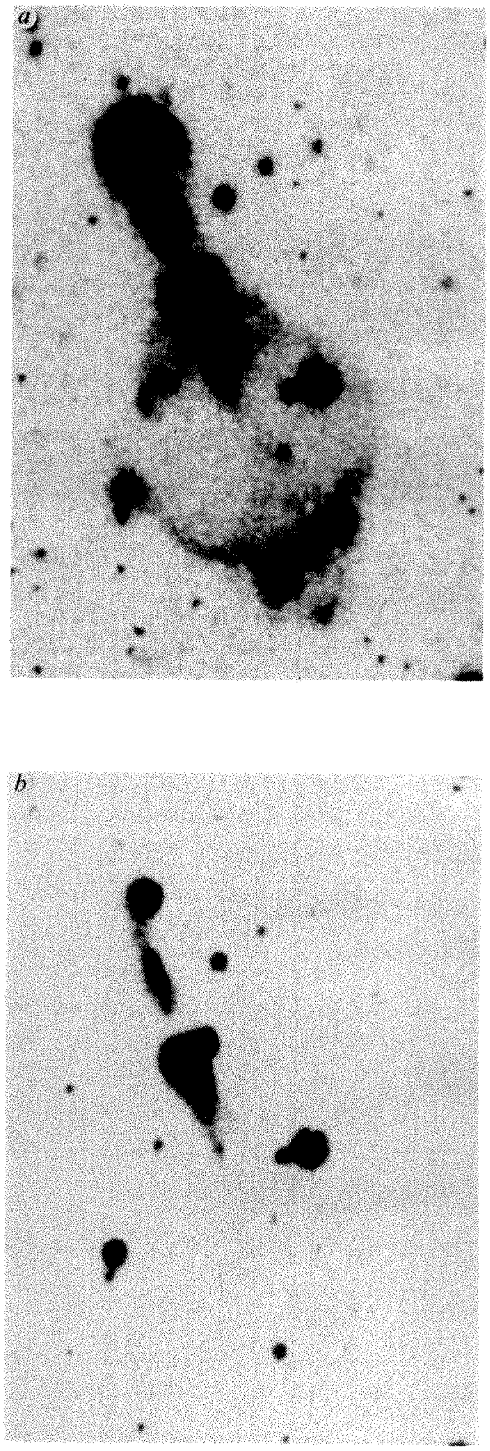

Fig. 1 CCD images of OH231.8+4.2 obtained with the Danish 1.5-m telescope at ESO, La Silla, through different interference filters, on 6 March 1986, both 60-min exposures. *a*, Hα/[N II] image centred on 6,565 Å and *b*, continuum image centred on 6,646 Å, both with FWHM (full width at half maximum) bandwidth of 78 Å. The images demonstrate that the bubble structures are purely line-emission objects. The total extent of the object is 50 arc s. North is up and east is left.

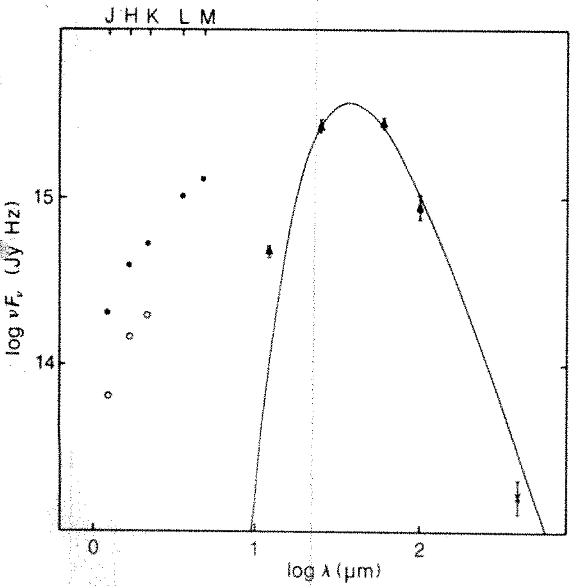

Fig. 2 The energy distribution of OH231.8+4.2. Filled circles are observations from this paper through a 15-arc s diaphragm, close to maximum light. Open circles are from ref. 4, through a 36-arc s diaphragm, on JD 2444673, close to minimum light. JHKLM denote the 1.25 to 4.8 μm photometric bands listed in Table 1. Triangles are fluxes from the IRAS Point Source Catalogue. The IRAS fluxes as plotted are approximate, because (1), the object is listed as variable; (2), the object is listed as a small extended source; and (3), no colour-correction factors were applied. The cross is a 400-μm measurement by Sopka *et al.*[8]. Error bars of the near-infrared measurements are less than the extent of the circles. The solid line represents a black body at a temperature of 100 K.

12 and 400 μm of about 2,700 $L_\odot$. Much of this large luminosity is from dust heated to ~100 K.

Part of this dust may be visible as the dark obscuring lane separating the northern and southern lobes in the CCD images. This is evidently a direct optical detection of a circumstellar disk. The star must be situated inside the disk, buried at the very base of the narrow northern lobe, because the northern flow axis is inclined towards us. Therefore, the brightest spot seen in all CCD images as well as in the infrared maps of Allen *et al.*[1] is not the location of the star, but simply a part of a reflection nebula, which fades to the south because of increasing distance from the illuminating source, and fades to the north because of increasing extinction in the disk. The absolute position of the star is known from the OH observations, but cannot be compared with the CCD images, for which astrometric positions have not been obtained. However, the infrared position was determined by Allen *et al.*[1], and does not coincide with the stellar position derived from the maser observations, but is in fact displaced 2 arc s southwards along the flow-axis. In the 2 μm map of Allen *et al.*[1], the intensity decreases towards the star over this distance by a factor of ten, which corresponds to an increase in extinction of 2.5 mag at K, if the underlying emission is constant, but the underlying emission certainly increases strongly towards the star, and the above value is therefore very much a lower limit. Assuming a normal reddening law (not necessarily a good assumption in a circumstellar disk) this then corresponds to an increase in visual extinction along the disk radius ≥20 mag.

Figure 3 shows the interpretation discussed above. The contours are drawn from the Hα/[N II] image shown in Fig. 1*a*, and all field stars are shown as filled circles. The distance from the centre of the disk to the centre of the brightest contour in the southern lobe is about 3 arc s (larger than in the 2-μm map, as expected from the higher extinction in the optical). With the disk inclined about 47° to the line of sight and at a distance of 1.2 kpc, this suggests a lower limit to the disk radius of ~5,000 AU. This compares with the radius of ≥6,000 AU determined from the ring of OH masers surrounding the embedded star[5].

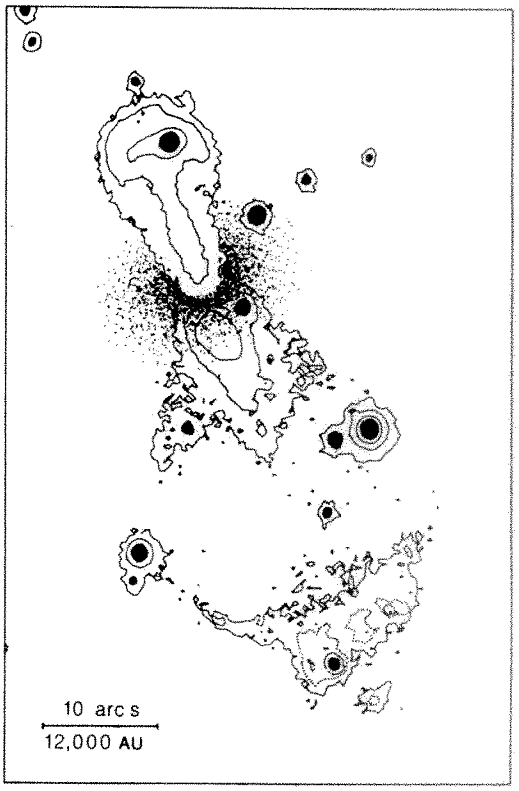

Fig. 3 A schematic representation of OH231.8+4.2, based on a contour plot of the Hα/[N II] CCD image in Fig. 1*a*. A disk surrounds the central star, which is invisible along our line of sight. Collimated bipolar outflows emerge above and below the disk. The surface brightness of the luminous southern lobe decreases towards the star because of extinction in the disk. A similar, but much less pronounced effect is seen in the narrow northern lobe, proving that the dust envelope is indeed primarily a highly flattened disk structure and not a near-spherical envelope. This is also indicated by the 'horns' or two-fingered appearance of the second brightest contour in the southern lobe, because Morris[9] has shown that when the dust density falls off rapidly with increasing angle from the equatorial plane, such a geometry occurs. Shocked bubbles are formed by collimated flows emerging from the central source and ramming into the ambient medium. Contour levels were chosen to outline important features and are in arbitrary units 285, 330, 490, 800, with a background level of about 265. The HH knots in the southern bow shock are inserted as dotted contours at a level of 305. Stars are shown as black circles, and cosmic rays (seen in Fig. 1*a*) have been erased.

The shocked bubbles around OH231.8+4.2 are obviously formed by a collimated outflow that rams into the ambient medium. This medium must be denser than the general interstellar medium for the two polar streams to produce the large and bright shock structures. Rather, the present collimated high-velocity wind is probably encountering a previous, slow, low-density outflow, producing the shocked emission at the interface zone. Indeed, from the clear limb-brightening of the southern lobe it is obvious that it is not filled with a uniform, luminous shocked gas, but that the shocks occur in a thin layer at the bubble boundary. The bow-shock of the northern lobe appears rather smooth within the present resolution, whereas the southern bow shock shows three condensations, with another isolated knot below them. These HH objects are apparently formed as a dynamical instability or a cooling instability in the bow-shock. The northern bubble is obviously less developed than the southern. This could either be because of a density-gradient in the older, low-density wind, through which the present flow rams, or an asymmetry in the collimating disk. It might also be an intrinsic property of the bipolar outflow itself. Very high-resolution molecular observations may resolve this. The reason that the most dense circumstellar material is located in a disk is perhaps related to the central star being a binary star[7,10]. The flow itself may be driven by radiation pressure on dust grains, a mechanism suggested to drive mass loss from highly evolved red-giant stars. The fact that the momentum flux in the flow from OH231.8+4.2 is roughly an order of magnitude larger than the momentum flux in the present stellar radiation[11,12] does not necessarily violate this hypothesis, because the star is clearly in a rapidly developing phase, as also suggested by the change from a slow to a fast wind[13,14].

The next stage for OH231.8+4.2 is presumably the formation of a planetary nebula. At the end of the heavy mass-loss phase, the ultraviolet radiation from the then naked stellar core will ionize the ambient medium. Unless the massive disk has been completely dispersed at that time (which seems unlikely), the shadow effect of the remaining disk material will cause a bipolar geometry of the planetary nebula. A radio continuum search with the VLA (very large array) shows that OH231.8+4.2 has not yet developed a directly observable ionized core[15].

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Neutrino oscillations in the Earth suggest a terrestrial test of solution to solar neutrino problem

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It is generally believed that a complete spectroscopy of solar neutrinos, which is far from being achieved in the near future, is required in order to verify the Mikheyev–Smirnov–Wolfenstein (MSW) solution[1,2] of the solar neutrino problem[3,4] and to unfold the values of neutrino masses and mixings that nature has selected. However, as we discuss here, the MSW solution[1,2] of the solar neutrino problem[3,4] can be tested with present equipment—the 'Bethe solution'[5] yields sizeable oscillations of atmospheric neutrinos in the Earth which can be detected with the massive underground proton decay detectors, while the 'Rosen-Gelb' additional solutions[6] yield diurnal and seasonal modulations of the solar neutrino flux which perhaps can be detected by the radiochemical Cl and Ga detectors. Moreover, neutrino oscillations in Earth may modify the values of the oscillation parameters which can solve the solar neutrino problem and help determine their values.

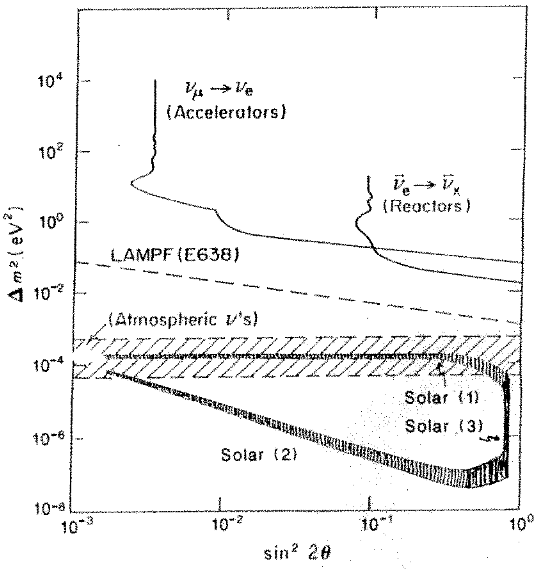

Fig. 1. Summary of experimental results on Δm^2 and $\sin^2 2\theta$. The areas to the right hand side of the full lines are excluded by neutrino oscillation experiments at accelerators and reactors. The area above the dashed line will be tested within a few years at Los Alamos Meson Production Facility (LAMPF). The shaded area can be tested by searching oscillations of atmospheric neutrinos with $0.1 \leq E_\nu \infty$ GeV. The narrow black strips indicate the values that can solve the solar neutrino problem[1,5-7].

The 'solar neutrino problem' consists of the well-known discrepancy between the expected production rate of ^{37}Ar atoms by solar neutrinos through the reaction $\nu_e + {}^{37}\text{Cl} \rightarrow e^- + {}^{37}\text{Ar}$, and the production rate that has been observed by R. Davis and collaborators since 1970. Whereas the standard solar model with the best available input physics predicts a neutrino capture rate of $7.6 \pm 3.3 (3\sigma)$ SNU (refs 3, 4) the observed capture rate is only 2.1 ± 0.3 SNU (refs 3, 4) (1 SNU $= 10^{-36}$ captures per ^{37}Cl atom per second). In spite of many attempts no simple explanation for this discrepancy has been found[3]. However, recently Mikheyev and Smirnov[1] offered a plausible solution to the solar neutrino problem. They found by numerical integration of the neutrino propagation equations[2] in the Sun that thermonuclear ν_es which are produced in the core of the Sun can convert into ν_μs before emerging from the Sun. These escape detection in the chlorine solar neutrino detector of R. Davis because their energy $E_\nu \leq 14$ MeV, is below the threshold energy for the reaction $\nu_\mu + {}^{37}\text{Cl} \rightarrow \mu^- + {}^{37}\text{Ar}$.

The discovery of Mikheyev and Smirnov has been confirmed by independent calculations by Bethe[5], by Rosen and Gelb[6] and by Dar *et al.*[7]. The detailed analysis[1,5-7] shows that neutrino oscillations in the Sun can solve the solar neutrino problem only if nature has selected specific combinations of neutrino masses and mixing angle. If ν_e and ν_μ, the neutrino flavour eigenstates which are produced in the weak interaction, are not eigenstates of the free hamiltonian but a linear combination of the mass eigenstates ν_1 and ν_2 with masses m_1 and m_2 respectively, that is $\nu_e = \cos\theta\nu_1 + \sin\theta\nu_2$ and $\nu_\mu = \cos\theta\nu_2 - \sin\theta\nu_1$, then the solar neutrino problem can occur because of neutrino oscillations in the Sun if the masses and mixing satisfy either[1,5-7]

(1) the Bethe condition[5]: $\Delta m^2 \cos 2\theta \sim 1.2 \times 10^{-4}$ eV^2 and $\tan^2 2\theta > 10^{-3}$, or

(2) the Rosen-Gelb condition[6]: $\Delta m^2 \sin^2 2\theta / \cos 2\theta \sim 4 \times 10^{-8}$ eV^2 and $\tan^2 2\theta > 10^{-3}$, or

(3) the condition[7]: $1 \times 10^{-4} \leq \Delta m^2 \leq 4 \times 10^{-8}$ eV^2 and $\sin^2 2\theta \sim 2/3$,

where $\Delta m^2 = m_2^2 - m_1^2$. Conditions (1)-(3) are indicated in Fig. 1.

If nature has selected neutrino masses and mixing angles that satisfy condition (1) then the pp neutrinos do not convert into

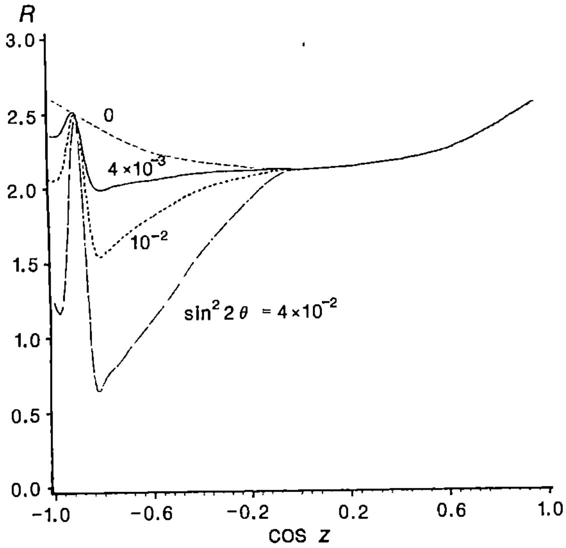

Fig. 2 The expected ratios between atmospheric ν_μs and atmospheric ν_es as function of zenith angles, for $E_\nu \sim 300$ MeV, $\Delta m^2 = 1.2\times10^{-4}$ eV^2, and for various values of $\sin^2 2\theta$.

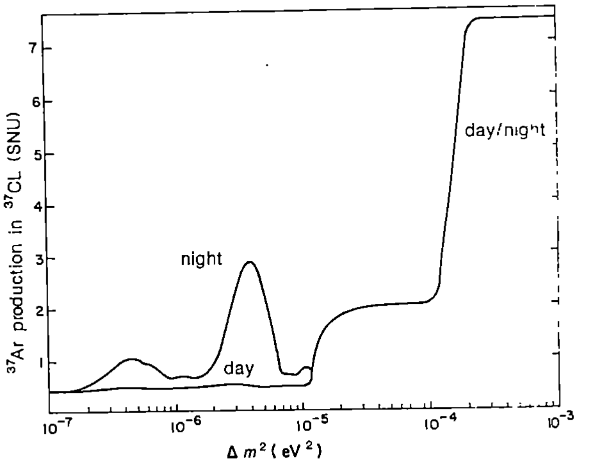

Fig. 3 The production rate of ^{37}Ar in ^{37}Cl at latitude 43° N as function of Δm^2 during day-time and during night-time, by solar neutrinos, for $\sin^2 2\theta = 0.10$.

ν'_μs in the Sun and the production rate of ^{71}Ge in ^{71}Ga should be that predicted by the standard solar model. In this case verification of the MSW solution of the solar neutrino problem in Cl cannot come from the Ga experiments[8-10]. It can, however, come from detecting sizeable oscillations of atmospheric neutrinos with $E_\nu \sim 300$ MeV in Earth, using the current massive underground proton decay detectors as neutrino telescopes (or from measuring the ratio between charge exchange reactions induced by solar ν'_es and the elastic scattering of both solar ν'_es and solar ν'_μs with (future) second generation proton decay detectors such as Kamiokande II, IMB II, and Icarus).

To see that let us consider the propagation of ν'_es and ν'_μs through Earth. In terms of Pauli's spin matrices σ_i[7]

$$\frac{\mathrm{d}}{\mathrm{d}x}\begin{pmatrix}\nu_e\\ \nu_\mu\end{pmatrix} = \mathrm{i}\,\frac{\pi}{l_v}(\alpha\sigma_1+\gamma\sigma_3)\begin{pmatrix}\nu_e\\ \nu_\mu\end{pmatrix}, \tag{1}$$

with $\alpha = \sin 2\theta$ and $\gamma = l_v/l - \cos 2\theta$ where,

$$l_v = 4\pi E_\nu/\Delta m^2 = 2.48\times E(\mathrm{MeV})/\Delta m^2\,\mathrm{eV}^2)\ \mathrm{cm} \tag{2}$$

is the oscillation length in vacuum and[5-7]

$$l = \sqrt{2}\pi/N_e G_F = 1.64\times10^9\ \mathrm{cm}/\rho_e \tag{3}$$

is the refraction length in matter, G_F is the Fermi coupling constant and ρ_e is the electron density divided by Avogadro's number; $\rho_e = N_e(\mathrm{cm}^{-3})/6\times10^{23}$.

In a medium of constant density, equation (1) can be solved analytically by simple exponentiation. Using the algebra of the Pauli spin matrices one obtains

$$\begin{pmatrix}\nu_e\\ \nu_\mu\end{pmatrix}_x = [\cos(\pi x/l_m) + \mathrm{i}(l_m/l_v)\sin(\pi x/l_m)(\alpha\sigma_1+\gamma\sigma_3)]\begin{pmatrix}\nu_e\\ \nu_\mu\end{pmatrix}_0 \tag{4}$$

Note in particular that the probability of neutrino flavour flip is given by[2]

$$P(\nu_e \leftrightarrow \nu_\mu, x) = (l_m/l_v)^2 \sin^2 2\theta\cdot\sin^2(\pi x/l_m), \tag{5}$$

$$l_m = l_v/(\sin^2 2\theta + (\cos 2\theta - l_v/l)^2)^{1/2}, \tag{6}$$

where l_m is the oscillation length in matter. $P(\nu_e \leftrightarrow \nu_\mu)$ as a function of l_v, that is as a function of $E_\nu/\Delta m^2$, has a resonance shape around $l_v = l\cdot\cos 2\theta$ whose height is $\sin^2(\pi x \sin 2\theta/l_v)$ and its full width at half the height is $\Delta l_v = 2l\cdot\sin 2\theta$.

For mixing angles which are not too small, equations (4)-(6) describe well[7] the propagation within the mantle and the core of Earth if one uses the average densities there instead of the exact density distributions[11]. In the general case when the electron density changes along the neutrino trajectory equation (4) can be used to propagate the neutrinos in small steps[7]. We have used equation (4) and the density distribution of Earth from ref. 11 for calculating $P(\nu_e \leftrightarrow \nu_\mu)$, the probability of a neutrino flavour flip after crossing Earth. Our calculations show that a significant probability of neutrino flavour flip develops for $\sin^2 2\theta \geqslant 10^{-2}$ and zenith angles z satisfying $\cos z \leqslant -0.25$, for neutrinos which satisfy the resonance condition in Earth: the electron density of Earth changes from $\rho_e \sim 1.3$ near its surface to about $\rho_e \sim 6.0$ near its centre[11]. Thus, the resonance condition $l_\nu = l\cdot\cos 2\theta$ in Earth is satisfied by neutrinos with $10^6 \leqslant E/\Delta m^2 \leqslant 5\times10^6$ MeV/eV^2, that is by neutrinos with $120 \leqslant E \leqslant 600$ MeV if condition (1), $\Delta m^2 = 1.2\times10^{-4}$ eV^2, is satisfied.

The expected ratio between ν_μs and ν_es that reach the underground detector from below at zenith angle $z \geqslant \pi/2$ can be related to their ratio when they enter the Earth from the atmosphere at zenith angle $\pi - z$ on the far side of the Earth:

$$R \equiv (N_{\nu_\mu}/N_{\nu_e})_z = [(1-P)\cdot R + P]/[P\cdot R + (1-P)]_{\pi - z} \tag{7}$$

However, because geomagnetic and solar wind effects on the ratio ν_e/ν_μ are quite negligible, equation (7) can be tested with experimental data obtained at the same site and the experimental results at different sites can be combined to increase statistics.

In Fig. 2 we plotted R as function of zenith angle for atmospheric neutrinos with $E_\nu = 300$ MeV for representative values of $\sin^2 2\theta(4\times10^{-2}, 1\times10^{-2}, 4\times10^{-3}$ and 0). The ratio ν_μ/ν_e for downgoing atmospheric neutrinos was taken from the calculations of Dar[12] which agree well both with experiments and with other independent calculations[13]. As can be seen from Fig. 2 the effect of neutrino oscillations on R for $\Delta m^2 = 1.2\times10^{-4}$ eV^2 is quite large for values of $\sin^2 2\theta$ which are not too small. We believe that such an effect, if indeed present, can be detected by the massive underground proton decay detectors because (i) the observed rate of interactions of atmospheric neutrinos in the proton decay detectors peaks around 300 MeV, which is the resonance energy in the Earth mantle of neutrinos that satisfy condition (1), (ii) the ratio N_{ν_μ}/N_{ν_e} is known[14] to a precision of about 5%, (iii) the 'contamination' of the interactions of ν_es and ν_μs by antineutrinos is a small effect. (The fluxes of atmospheric antineutrinos are smaller and their cross sections are much smaller than those of neutrinos.) Therefore we suggest that the data on interactions of atmospheric neutrinos in massive underground proton decay detectors will be re-analysed to see whether there is any evidence for the 'Bethe solution'[5].

If nature has selected neutrino masses and mixing angles that satisfy condition (2), or (3), then the rate of production of ^{71}Ge in ^{71}Ga decreases vastly from 126 SNU (73 SNU due to pp

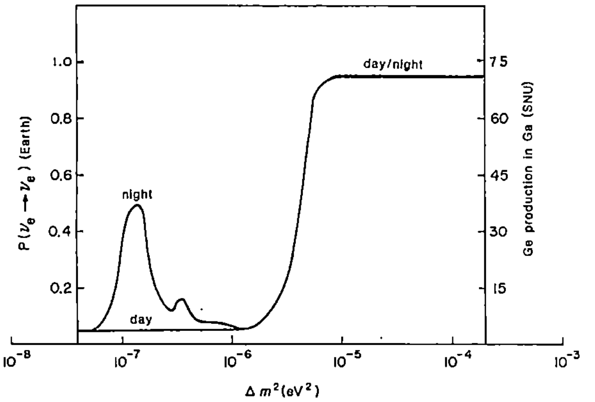

Fig. 4 The probability that pp neutrinos from the Sun reach a terrestrial detector at latitude 43° N as electron neutrinos, during day-time and during night-time, as function of Δm^2, for $\sin^2 2\theta = 0.1$, and the corresponding rate of ^{71}Ge production in ^{71}Ga.

neutrinos) for $\Delta m > 10^{-5}$ eV^2 to practically zero for $\Delta m\ 10^{-6} <$ eV^2, as shown in Fig. 4 by the 'day' line. But even if the Ga experiments do detect a signal much smaller than expected from the standard solar model and consistent with the MSW effect, it will neither verify the MSW effect nor will it establish the solar origin of the signals in the Cl or the Ga experiments. The expected capture rate for the decay hypothesis is indistinguishable[7] from the expectation based upon the MSW effect with parameters satisfying condition (2).

But the estimated effects of the MSW solution on the Cl and the Ga experiments neglected[1,5-7] neutrino oscillations in the Earth. At night solar neutrinos cross the Earth before reaching the detector. Passage through the Earth induces $\nu_e \leftrightarrow \nu_\mu$ transitions. Because the transition probability depends on the path in Earth, that is, on the position of the Sun relative to the detector, then neutrino oscillations in Earth may induce both diurnal and seasonal modulations of the production rates in radiochemical detectors and changes in the rate and in the spectrum of recoiling electrons in electron-recoil detectors.

The zenith angle of the Sun, z, as function of time at a geographical latitude ϕ ($\phi \sim 43°$ N for Homestake, Mont Blanc, Frejus, Grand Sasso and Baksan) is given by

$$\cos z = \sin\phi \sin\delta - \cos\phi \cos\delta \cos(2\pi h/23.934), \qquad (8)$$

where h is the day time (in hours), 23.934 h is the mean sidereal day and δ is the Sun's declination as a function of time. $\sin\delta \approx -\sin(23.44°)\cos(2\pi d/365.242)$, where 23.44° is the angle at which the ecliptic is declined to the plane of the celestial equator, 365.242 is the length in days of a tropical year and d is the time in the year (in days).

To estimate the diurnal effect we used equation (8) to perform the time averaging of $P(\nu_e \leftrightarrow \nu_\mu)$ which was calculated using equation (4) and the density distribution of Earth from ref. 11. The diurnal effect at latitude 43° N is demonstrated in Figs 3, 4 where we plotted the expected rates of ^{37}Ar production in ^{37}Cl, and of ^{71}Ge production in ^{71}Ga because of pp neutrinos, respectively, during day and night, as a function of Δm^2 for $\sin^2 2\theta = 0.1$. As can be seen from Figs 3, 4, if solar neutrinos are converted in the Sun into ν_μs then the Earth may transform a large fraction of them, during night-time, back into ν_es and the day-night difference in the radiochemical detectors may be quite large, (depending on the specific values of masses and mixing angles that nature has selected). Unfortunately, the ^{37}Ar in the ^{37}Cl experiment was extracted after exposures between 35 and 80 days (ref. 15 and R. Davis Jr, personal communication). To reveal a day-night modulation one will have to extract continuously the Ar atoms produced in Cl (the Ge atoms produced in Ga) into separate day and night containers and count them separately, which apparently can be done with reasonable efficiency (R. Davis Jr, personal communication). However, a day-night difference, if it is sufficiently large should reveal itself also in a summer-winter difference. At 43° N the ratio between the average time that the Sun is below the horizon in the winter (1 October-31 March) and in the summer (1 April-30 September) is 1.40. Since our calculations show that the time-averaged probabilities in winter nights and in summer nights are approximately equal, therefore at Homestake the ratio between the numbers of Ar atoms produced in Cl by solar neutrinos during the winter and during the summer should be between 1 and 1.40 (1.40 if the MSW effect in the Sun transforms all solar ν_es into ν_μs, less than this otherwise).

After this paper was submitted for publication three further papers have appeared on the topic of resonant amplification of oscillations of atmospheric and solar neutrinos in Earth[16-18].

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Icosahedral C$_{60}$: an aromatic molecule with a vanishingly small ring current magnetic susceptibility

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Smalley and co-workers[1] have speculated that an icosahedral C$_{60}$ molecule might have unusual magnetic properties. Here we report that the π-electron ring-current susceptibility of C$_{60}$ is unusually small and sensitively dependent on the relative strengths of the two inequivalent bonds of the molecule. For equal bond strengths the susceptibility is only −0.21 that of benzene (with field normal to the plane of the six-membered ring). A 2% change in relative bond strengths is enough to change the sign of the susceptibility with the result that C$_{60}$ is probably weakly diamagnetic. The shielding effect of the π-electrons is calculated to be less than 1 p.p.m. for an atom placed at the centre of C$_{60}$. A larger effect is expected for C$_{60}^{6-}$, which is calculated to be strongly diamagnetic.

Recently Smalley and co-workers[1-3] have produced evidence for the generation of C$_{60}$, which they formulated as a truncated icosahedron. Their suggestion that C$_{60}$ might provide the first example of a spheroidal aromatic molecule (Fig. 1) has been supported by π-electron and total energy calculations[4-10]. A further suggestion by Smalley and co-workers[1] which (in certain circumstances[11,12]) also bears on the aromatic character of C$_{60}$ has yet to receive theoretical attention. They noted that "The inner and outer surfaces are covered with a sea of π electrons" and went on to suggest that "... the chemical shift in the NMR

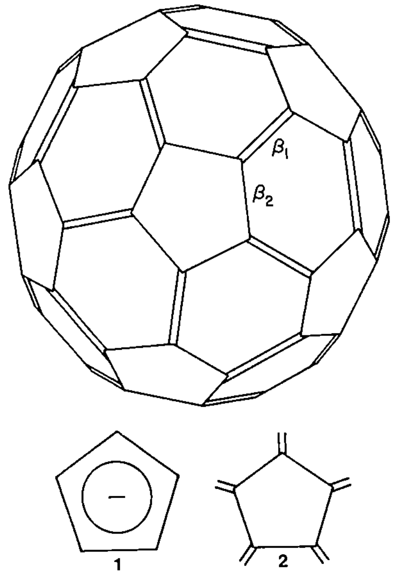

Fig. 1 Molecular structure of icosahedral C_{60}; cyclopentadienide anion (1); [5]radialene (2).

of the central atom [placed in the interior] should be remarkable because of the ring currents." This intriguing hypothesis is the subject of the present paper.

We have calculated the ring-current magnetic susceptibility of C_{60} with the finite-field version of the original London theory[13]. Adopting a common bond length (with resonance integral (taken to be positive) equal to that of benzene (β)), we find the π-electron ring current magnetic susceptibility of C_{60} to be −0.21 that of benzene ($\chi_\pi^{C_{60}}/\chi_\pi^{\text{benzene}}$ with field normal to the plane of the benzene six-membered ring (6-MR)). Thus the susceptibility of C_{60} is much smaller than that of benzene and is paramagnetic (but see below). $\chi_\pi^{C_{60}}$ is isotropic so the rotationally averaged (solution) value is unchanged from the static value, whereas $\chi_\pi^{\text{benzene}}$ is decreased threefold. Scaling the resonance integral in C_{60} for non-planarity (as in the 3-dimensional Hückel molecular orbital theory)[10,14] would further reduce χ_π.

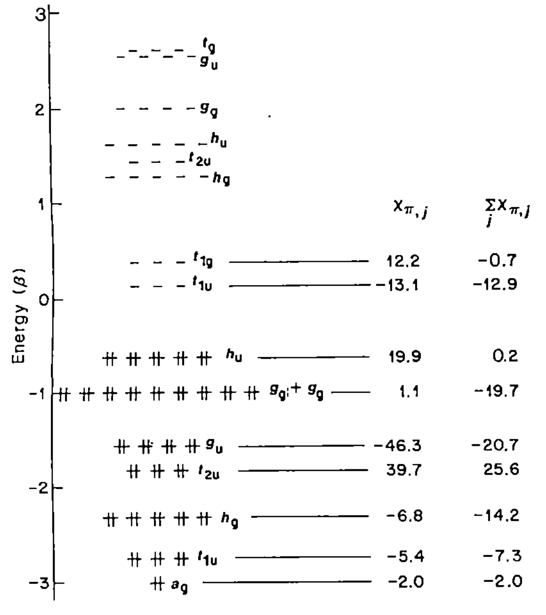

Fig. 2 Hückel molecular orbital[20], j, energy levels in units of β and π-orbital magnetic susceptibilities in units of $|\chi_\pi^{\text{benzene}}|$ calculated for C_{60} with a common resonance integral. Occupation of an orbital is denoted by a solid vertical line; labels refer to irreducible representations of the icosahedral point group.

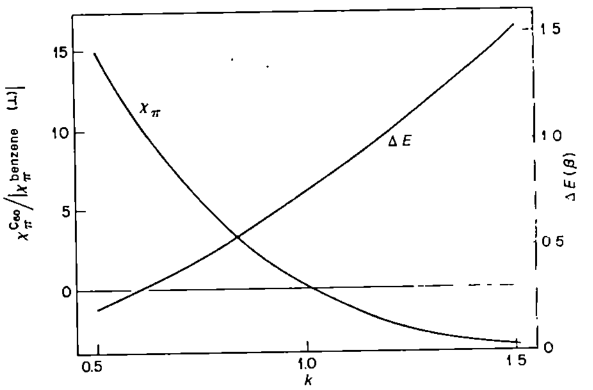

Fig. 3 π-Electron magnetic susceptibility as a function of the relative resonance integrals of C_{60}. Also shown is the behaviour of ΔE, the energy gap between the highest occupied and the lowest unoccupied molecular orbitals.

Figure 2 shows the contributions to the magnetic susceptibility by individual molecular orbitals ($\chi_{\pi,j}$). Many of the orbitals possess large susceptibilities, and the low molecular magnetic susceptibility is brought about by an essentially complete cancellation (see below). It has been predicted that C_{60} will have an exceptionally high electron affinity[10], and C_{60}^{6-} is calculated to be strongly diamagnetic and C_{60}^{12-} weakly diamagnetic (Fig. 1). This contrasts with planar aromatic hydrocarbons and graphite, which show appreciable diamagnetism in the neutral state and a very stong ring-current paramagnetism when reduced[15-19].

In a magnetically isotropic molecule, with π-electrons on the surface of a sphere, the ring-current contribution to the NMR chemical shift of a central atom takes the form $\delta_{RC} = 2\chi_\pi/r^3$, where r is the radius of the sphere and positive δ signifies deshielding. For C_{60} with a radius of $2.478l$ (where l is the carbon-carbon bond length, 1.4 Å), the relationship takes the form $\delta_{RC} = 0.0443\,(\chi_\pi^{C_{60}}/|\chi_\pi^{\text{benzene}}|)\,(\beta/\text{kcal mol}^{-1})$ p.p.m.. Using the two empirical parameterizations for β(61.4(A) and 50.4(B) kcal mol^{-1})[15], we obtain for the chemical shifts (respectively), δ_{RC} = 0.57, 0.46 p.p.m. (C_{60}); −35.0, −28.8 (C_{60}^{6-}); −1.9, −1.5 (C_{60}^{12-}).

Although all the atoms in C_{60} are related by symmetry, there are two types of bond: those at the junction of two 6-MRs (denoted by β_1) and those between a 5-MR and a 6-MR (β_2) (Fig. 1). If $\beta_1 = k\beta$ and $\beta_2 = \beta$, the ratio of the resonance integrals is given by $\beta_1/\beta_2 = k$. All the theoretical calculations find the 6/6-MR bonds to be shorter than the 5/6-MR bonds[4-10]. Thus $\beta_1 > \beta_2$ and, from the calculated bond lengths in the literature and an exponential dependence of the resonance integral on bond length[20], it is likely that $1.0 < k < 1.3$. Figure 3 shows χ_π for C_{60} as a function of k: χ_π changes sign in the vicinity of $k = 1.02$. Given the estimates[4-10] of the bond lengths in C_{60} it is likely that k is larger than this and that the ring-current susceptibility in C_{60} is weakly diamagnetic purely because of the relative bond strengths.

A useful reference point in understanding the completely unexpected magnetic properties of C_{60} is provided by the cyclopentadienide anion (1) and [5]radialene (2)[21]. For the monocyclic 5-MR systems 1 and 2 the relative susceptibilities are given by $\chi_\pi^2/\chi_\pi^1 = 0.0015$. Thus it would seem that the magnetic response of C_{60} is rather like that of [5]radialene. This point of view is complemented by the results in Fig. 3, where the paramagnetism increases as the energy gap (ΔE) decreases. The bond order in C_{60} is large and thus a strong Larmor diamagnetism is expected (see below), but this is apparently cancelled by substantial Van Vleck paramagnetism of order $1/\Delta E$[22,23].

As a final comparison, we consider the classical Pauling[24] free-electron treatment of the ring-current magnetic susceptibility of C_{60}. If the molecule is treated as a spherical surface of 60 π-electrons and the susceptibility calculated from the Larmor formula for free precession of the electrons in a magnetic field, we obtain $\chi_\pi = -60(e^2/4m_e c^2)\langle\rho^2\rangle$, where $\langle\rho^2\rangle$, the expectation value of the mean square radius of the electron orbits is given by $2r^2/3 = 4.09 l^2$. Thus the free electron theory gives $\chi_\pi^{C_{60}}/|\chi_\pi^{\text{benzene}}| = -(60/6)(4.09) = -40.9$ (strong diamagnetism). The quantum-mechanical London calculation presented earlier shows that the correct value is less than one-hundredth of the classical value.

Although planar aromatic hydrocarbons and graphite have provided appropriate points of analogy for the π-electron energy structure of C_{60}, it is clear that the magnetic response of C_{60} is unlike that of any other molecule yet encountered.

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A new method for measuring the surface energy of solids

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Although the surface tension of liquids has been understood and measured since the time of Young[1] and Laplace[2], the surface energy of solids has eluded understanding and evaded measurement, despite its postulated importance to catalysis, crystal growth, colloidal behaviour, sintering and fracture. It is salutary to reflect that solid surface energies are even now more readily determined by theoretical argument than by experiment. Here we show that solid surface energies can be evaluated experimentally by measuring the elastic modulus of submicrometre powder assemblies, knowing the particle diameter, elastic modulus and volume fraction.

The problem is that solids should display higher surface energies than liquids, but their atoms are relatively immobile, so that stresses in the surface may exist in addition to the surface tension of the solid. Attempts to distinguish the genuine solid surface tension from such spurious stresses have not been satisfactory[3,4]. For the same reason experiments to find the surface energy of the solid by measuring the enhanced vapour pressure[5], increased solubility[6], enlarged heat of dissolution[7] or lowered melting point[8] of powders have had only limited success; other energy terms shroud the surface contribution to such an extent that the very existence of solid surface energy has been doubted[5].

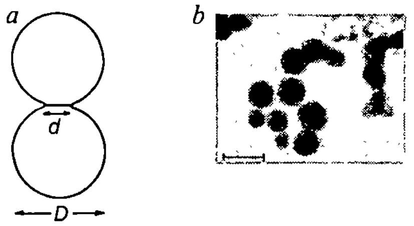

Fig. 1 *a*, Two elastic, smooth spheres in contact under zero external load form a contact spot of diameter *d*. *b*, Transmission electron micrograph of silica particles (OX50, Degussa). Scale bar, 0.1 μm.

Similar obstacles have dogged the cleavage method, the most popular way of estimating solid surface energies since Griffith[9] proposed his criterion of brittle fracture and Obreimoff[10] showed the importance of gaseous contamination. Although cleavage experiments have given results close to the theoretically expected values[11], it is more usual to obtain inordinately high figures because plastic flow occurs under the high stresses near the crack tip, even for brittle materials, causing a large and indeterminate energy demand[12].

Our new method avoids this difficulty because it is an equilibrium measurement which does not involve cleavage. Instead it is a development of the method devised for rubber, measuring the equilibrium diameter of the contact between two solid bodies held together by surface forces[13]. The interfacial energy between two plane surfaces of unit area was defined as Γ, the energy released on bringing the surfaces from infinity into contact. For identical solids, Γ was twice the surface energy of each piece, $\Gamma = 2\gamma$.

Experimentally, it proved better to use spheres rather than plane surfaces and to measure the enlargement of the Hertz[14] elastic contact circle by the attractive surface forces[15–19]. The interface energy Γ was then calculated from the equation

$$d^3 = 3(1-\nu^2)\frac{D}{E}\left[W + \frac{3}{4}\pi D\Gamma + \sqrt{\frac{3}{2}\pi DW\Gamma + \left(\frac{3}{4}\pi D\Gamma\right)^2}\,\right] \quad (1)$$

where d was the contact spot diameter, W the external load on the spheres, D the sphere diameter, E its Young's modulus and ν its Poisson's ratio. It was demonstrated that this method gave a better approach to equilibrium than a fracture test on the same geometry[13]. For 44 mm diameter rubber spheres the interfacial energy was measured to be $\Gamma = 71$ mJm^{-2} falling to 6.8 in water and to less than 1 mJm^{-2} in a 0.01 molar solution of sodium dodecyl sulphate. These values, inserted in Young's equation, were consistent with contact angle determinations. The main problem was surface roughness, inhibiting molecular contact and restricting the method to 'smooth' solids such as rubber or mica[20] which satisfied a smoothness criterion[21] depending largely on the centre line average roughness and the elastic modulus of the sample. Large spheres of ceramics would require almost atomically smooth surfaces to produce an adhesive contact.

In this report we recognize that this restriction of solid smoothness applies only to large bodies. It has previously not been observed that equation (1) should apply to all solids if the sphere size is made sufficiently small. In the limit, a single atom cannot be rough. Similarly, a 10 nm diameter particle should appear smooth as far as adhesion is concerned. Although the upper bound to this argument has not been defined (because Fuller and Tabor[21] only addressed the contact of plane surfaces, following Johnson[22]) it is reasonable to suppose that equation (1) should hold for ceramic grains less than 1 μm in size. Particles of this kind are found to adhere to a considerable degree[23]. The snag in using such particles to measure surface energy is that the contact spot is only tens of nm in diameter. Electron microscopy proved useful in defining the contact for metal and polymer latex spheres[24,25]. Here it is shown that the contact spot

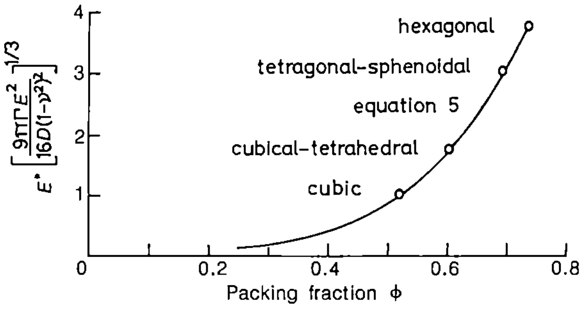

Fig. 2 Calculated elastic modulus for four regular sphere packings for comparison with equation (5).

size may be determined from the elastic modulus of a powder assembly[26] allowing the interfacial energy Γ to be calculated.

Consider two equal spheres in contact under zero external load (Fig. 1*a*). The diameter of the contact spot from equation (1) is

$$d=\left[\frac{9\pi}{2E}\Gamma D^2(1-\nu^2)\right]^{1/3} \tag{2}$$

This can be measured by applying a small test load P and following the elastic deformation δ of the spheres since[13]

$$P/\delta=Ed/2(1-\nu^2)=\left[\frac{9\pi\Gamma E^2D^2}{16(1-\nu^2)^2}\right]^{1/3} \tag{3}$$

Although the deformation at a single contact is minute, it can be magnified by stacking the spheres end to end in a regular cubic packing, whose elastic modulus E^* from equation (3) is

$$E^*=P/d\delta=\left[\frac{9\pi\Gamma E^2}{16D(1-\nu^2)^2}\right]^{1/3} \tag{4}$$

To judge the effect of changing the packing from simple cubic, the elastic modulus was also calculated for hexagonal close packing and for two other regular packings of uniform spheres. It was found that the elastic modulus increased with packing fraction ϕ (volume of spheres/volume of assembly) along a single curve (Fig. 2) described by

$$E^*=17.1\,\phi^4\left[\frac{\Gamma E^2}{D}\right]^{1/3} \tag{5}$$

Qualitatively, this ϕ^4 dependence of E may be understood in terms of two effects; first the ϕ^2 dependence of modulus on the density of particle packing[27]; second, a further ϕ^2 dependence of modulus on the coordination number of each sphere[28]. Based on this simple reasoning, it was proposed that irregular sphere packings should also fall on this same curve.

The following experiments were designed to test this hypothesis and then to use equation (5) to measure the interface energy Γ of ceramic particles. Silica (OX50, Degussa, $D=0.05\,\mu$m, $E=70$ GPa) (Fig. 1*b*) and titania (RCR2, Tioxide, $D=0.23\,\mu$m, $E=280$ GPa) were used to make the particle assemblies because they were nearly spherical and of relatively narrow size distribution. The particles were mixed in a polymer solution (30% polyvinyl alcohol (KH 17 s, Nippon Gohsei) in water) pressed to a sheet, dried, cut into strips, then heated to 500 °C to remove the polymer. The resulting particle assemblies were bend tested to determine Young's modulus E^* which was plotted against packing fraction ϕ derived from the weight and volume of the strip.

The measurements for titania (Fig. 3*a*) gave a good fit to the ϕ^4 relation of equation (5) and correspond to a value of $\Gamma=0.6\,\mathrm{Jm}^{-2}$ reasonably close to the theoeretical estimate of $\Gamma=0.8\,\mathrm{Jm}^{-2}$ for the 110 plane of rutile at 20 °C[29]. A powder compact made by pressing in a steel die gave a result near the curve, indicating that the modulus was not strongly affected by the preparation method. Possible errors in the experimental results stem from inaccuracy of particle diameter determination, the spread of particle sizes in the powder and anisotropy of particle shape and elasticity.

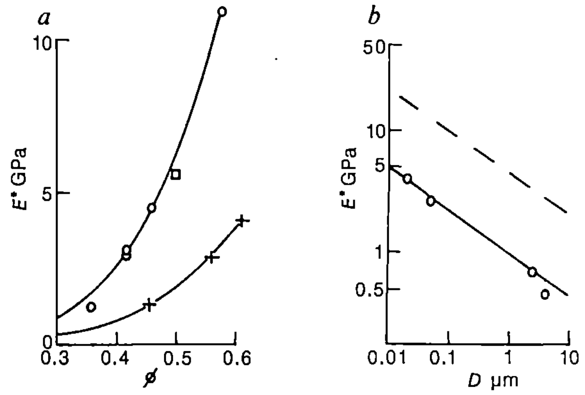

Fig. 3 *a*, Elastic modulus results for particle assemblies compared with equation (5). ○, Titania powder, mixed in polymer solution. □, Titania powder, die pressed, $\Gamma=0.6\,\mathrm{Jm}^{-2}$. +, Silica powder, $\Gamma=0.05\,\mathrm{Jm}^{-2}$. *b*, Results for silica powders of different particle diameter compared to equation (5). The broken line shows equation (5) plotted for $\Gamma=1.16\,\mathrm{Jm}^{-2}$, a theoretical estimate for pure silica[25]. The other line shows equation (5) plotted for $\Gamma=0.05\,\mathrm{Jm}^{-2}$.

Results for silica also fitted equation (5) but corresponded to $\Gamma=0.05\,\mathrm{Jm}^{-2}$, much lower than the theoretical $1.16\,\mathrm{Jm}^{-2}$ for the 110 plane of β cristobalite[29]. This low value was confirmed by additional measurements on a series of pure silica powders of different diameter, results which supported the $D^{-1/3}$ dependence of modulus (Fig. 3*b*). The low result for Γ was unchanged when the silica assembly was immersed in water, suggesting that the silica surface was already saturated by atmospheric contamination. Heating the silica to 700 °C gave a weight loss of almost 1% and increased the modulus by a factor of nearly 2, indicating that water was being driven off to increase the surface energy of the silica. On cooling and rewetting, the modulus fell back again. This observation is important to ceramics, which require low surface energy to ease powder compaction[30] but a high surface energy to drive sintering at elevated temperatures.

In conclusion, the surface energy of solids may be measured by determining the elastic modulus of fine powder assemblies, knowing the packing fraction, the diameter and the elastic properties of the grains. Although there are difficulties because many powders are not spherical, uniform or isotropic, it is believed that this new method may make the surface energy of solids as experimentally accessible as that of liquids, adding to our knowledge and application of surface phenomena.

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Mechanical instability of gels at the phase transition

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Polymer gels, consisting of a cross-linked polymer network immersed in liquid, undergo a volume phase transition: when external conditions such as temperature or solvent composition change, a gel reversibly swells or shrinks, but does so discontinuously[1–5]. The volume change at the transition can be as large as a factor of one thousand[2], and the phenomenon occurs in all gels[6,7]. The equilibrium aspects of the phase transition have been extensively studied, but its kinetics have not yet been fully explored. In particular, the appearance of patterns on the originally smooth surface of a gel during the transition makes the kinetic process difficult to understand. Here we elucidate the physical basis underlying the formation and evolution of the pattern.

When a polymer gel undergoes an extensive swelling, a beautiful, regular pattern appears on the surface of the gel (as shown on this week's cover). At the beginning, the pattern is extremely fine, having a texture similar to that of a frosted glass. As time goes on, the units of pattern coalesce, doubling their characteristic size. When the unit size becomes comparable to the size of the gel, the pattern gradually disappears. As the gel approaches equilibrium, it regains the shape identical to the original one. Finally the gel becomes totally homogeneous, and the process comes to an end.

The pattern is formed in all the gels we have studied, that is, some polyvinyl gels and gels made of natural polymers. We chose ionized acrylamide gels because of their relative ease of handling. Copolymeric gels of acrylamide (700 mM) and sodium acrylate (0-32 mM) are prepared by a standard method using free radical polymerization initiated by ammonium persulphate and catalysed by tetramethylethylenediamine[1]. The osmotic pressure of counterions from sodium acrylate exerts an internal osmotic pressure and causes the gel to expand when placed in water. The volume expansion increases with the amount of sodium acrylate. The gel has a radius ~2 cm before swelling and 8 cm after complete swelling. The gel has a perfectly smooth surface before swelling, and as the gel is placed in water an extremely fine pattern appears on the surface[8]. As time goes on, the unit size of the pattern becomes larger by coalescence of units into a larger unit, which is accomplished by conversion of side lines into thorns followed by the disappearance of thorns. Eventually the size of the unit becomes comparable to that of the gel radius, when the pattern disappears completely. It continues to swell to the final volume until it regains the spherical shape and totally homogeneous density.

Careful observation shows that the pattern consists of numerous line segments of cusps into the gel. These cusps are not due to breaking of the gel, nor are the representative of a shrunken portion of the gel. The line is a result of shear bending of a homogeneously swollen gel surface. Indeed, when the surface is sliced off in a thin layer, the pattern disappears instantly, and the slice becomes homogeneous. If the cusps were due to local shrinking of the gel, they would take a long time to disappear through the collective diffusion process. At each swelling stage, the pattern appears to be a hexagonal lattice, but it is quite irregular. A similar pattern is observed on the surface of the acrylamide gel formed in a Petri dish and swollen in water (Fig. 1). The patterns are formed only when swelling is extensive: if the swelling is small, the surface of the gel remains smooth throughout the entire process.

The pattern formation may be qualitatively explained in the following way. The kinetic process of the swelling of a gel is governed by the collective diffusion of the polymer network into a solvent[9]. At first, a very thin surface layer is swollen. This layer is under a mechanical constraint, namely, the outer surface of the layer is free to expand, whereas the inner surface is fixed to the core of the gel. Thus, the layer is under opposing demands on the upper and lower surfaces, one to expand and the other to remain unswollen. When the osmotic pressure is small, these opposing forces are resolved by stretching the gel unidirectionally perpendicular to the surface. When the osmotic pressure is large the outer surface is forced to buckle. The characteristic wavelength of the pattern must be proportional to the thickness

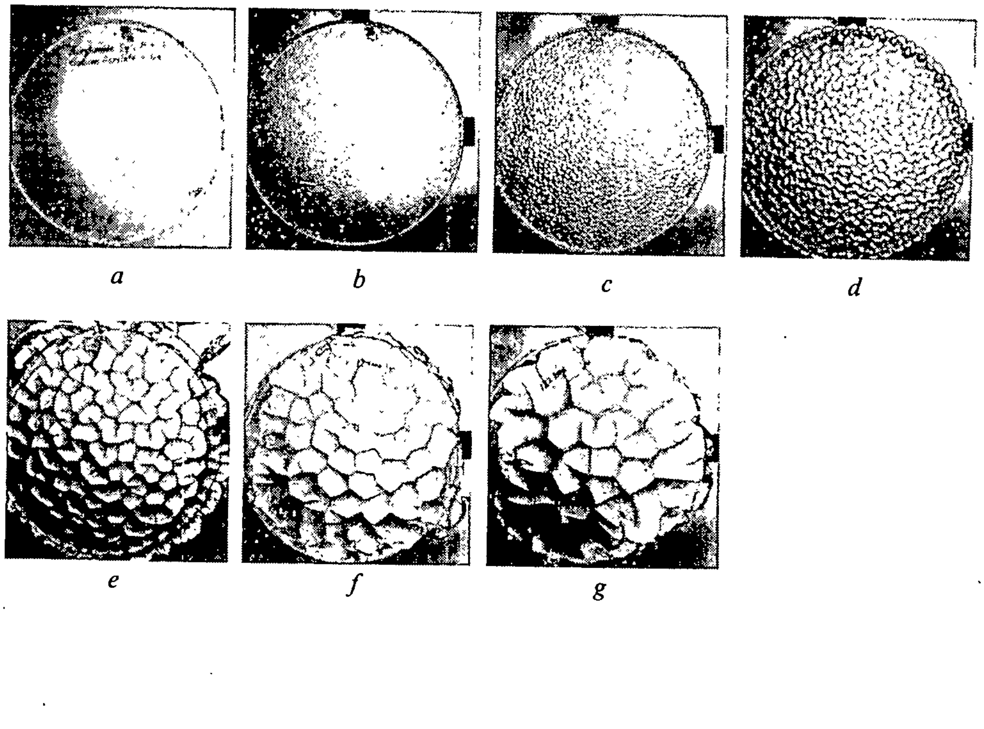

Fig. 1 An ionized acrylamide gel formed in a Petri-dish is allowed to swell in water. An extremely fine pattern appears on the free surface of the disk gel, and evolves with time ($a \to g$).

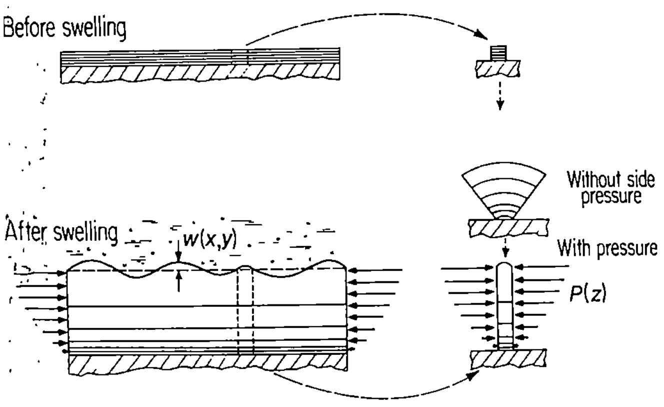

Fig. 2 The model of a gel on which the elastic deformation energy is calculated in the text. The gel slab is treated as a combination of thin layers. The lowest layer is mechanically fixed to a film, but the top layer is free to expand except for its boundary, which is fixed only in the horizontal direction.

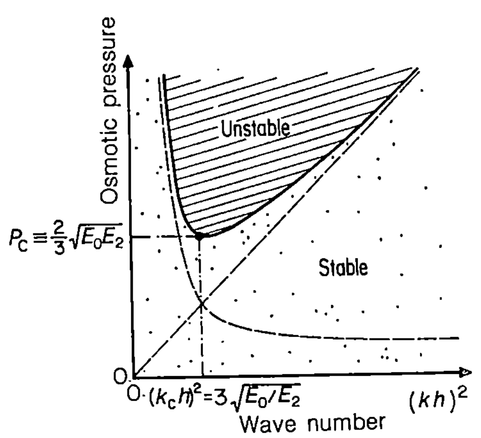

Fig. 3 The condition for instability is plotted for two independent parameters. The vertical axis represents the osmotic pressure and the horizontal axis denotes the wave-number of the fluctuations. The boundary for the instability depends on Young's modulus and the gel thickness.

of the swollen layer, as that is the only relevant length scale. As time goes on, the thickness of the swollen layer increases, as does the wavelength of the pattern.

To test the hypothesis presented above, we have carried out several demonstrative experiments. Gel slabs of thickness from 0.2–5 mm are prepared with one surface free, and the other surface covalently cross-linked to a film whose surface has polymerization-active chemical groups. When the gel slab is immersed in water, a pattern is formed and evolves in a similar way to the swelling of the spherical gel as shown before. But the evolution stops because the mechanical constraint is permanent. The final wavelength is proportional to the thickness of the gel slab.

We have further investigated the effect of internal osmotic pressure of the gel on the pattern formation. The relative amount of sodium acrylate is used to vary the osmotic pressure of counterions. There is a critical osmotic pressure above which distinct patterns appear, and below which the gel surface remains smooth, and this pressure is independent of the gel thickness.

We now theoretically analyse the phenomena using a logic similar to that introduced by Euler to describe the mechanical instability of a rod under a compressional force[10]. Consider a square slab of gel, with its upper surface free, but its lower surface mechanically fixed (see Fig. 2). When the gel is swollen the local degree of swelling monotonically increases from the fixed surface to the other free surface. The compressional pressure is a maximum at the top and decreases monotonically towards the lower surface. Let us assume that there appears a sinusoidal fluctuation in the gel, represented by a vertical displacement $\Delta z = w(x, y, z)$, where we define the coordinate system so that the z-axis is perpendicular to the gel surface. Assume further that the displacement changes monotonically with z. Then

$$w(x, y, z) = w(x, y)\sin\left(\frac{\pi z}{2h}\right) \qquad (1)$$

The mechanical potential energy of the gel associated with this shear deformation is given by

$$V = \iiint \frac{1}{2}\left\{\frac{Ez^2}{9}(\Delta w)^2 - P(\nabla w)^2 + \frac{E}{h^2}w^2\right\}\mathrm{d}\mathbf{r} \qquad (2)$$

The first term represents the bending energy of the gel, the second term the energy due to compression, and the last term represents the energy associated with the local vertical stretching. By Fourier-transforming the displacement

$$w(x, y) = \sum A_{\mathbf{k}}\,\mathrm{e}^{i\mathbf{k}\mathbf{r}} \qquad (3)$$

the mechanical potential energy becomes a sum of independent modes.

$$V = \frac{L^2h}{2}\sum\left\{\frac{E_2}{9}(kh)^4 - P(kh)^2 + E_0\right\}A_{\mathbf{k}}A_{-\mathbf{k}} \qquad (4)$$

where

$$E_i = \int E(z)\left(\frac{z}{h}\right)^i \sin^2\left(\frac{\pi z}{2h}\right)\frac{\mathrm{d}z}{h} \quad (i=0,2) \qquad (5)$$

and

$$P = \int P(z)\sin^2\left(\frac{\pi z}{2h}\right)\frac{\mathrm{d}z}{h} \qquad (6)$$

Modes having a negative value for the term in brackets in equation (4) become unstable, because then the increase in amplitude $A_{\mathbf{k}}A_{-\mathbf{k}}$ leads to lower energy. The instability condition

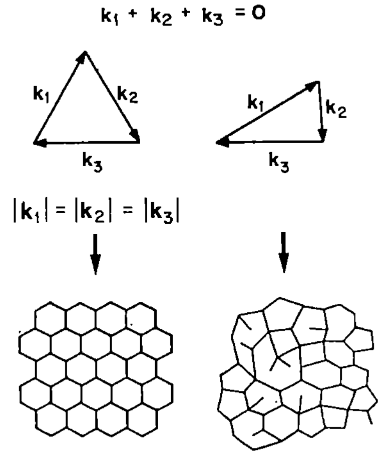
$\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3 = 0$
$\mathbf{k}_1$
$\mathbf{k}_2$
$\mathbf{k}_3$
$|\mathbf{k}_1| = |\mathbf{k}_2| = |\mathbf{k}_3|$

Fig. 4 A wave consisting of three standing plane waves having wave vectors $\mathbf{k}_1$, $\mathbf{k}_2$ and $\mathbf{k}_3$ of the same magnitude, which satisfy $\mathbf{k}_1+\mathbf{k}_2+\mathbf{k}_3=0$ constructs a hexagonal pattern. This situation is similar to the instability problem of the hexagonal Benard cells observed in a fluid layer heated from the bottom[11]. In the actual observation, however, the pattern is found to be not exactly hexagonal. Because the osmotic pressure is higher than the critical value P_c, more than one mode become unstable and grow at the same time. A combination of three modes having different wave numbers can now construct a closed, deformed triangular relationship. In such cases the pattern may be distorted from the perfect hexagonal pattern.

is given by

$$P > E_2\frac{(kh)^2}{9} + E_0\frac{1}{(kh)^2}$$

$$= \left(E_2^{1/2}\frac{kh}{3} - E_0^{1/2}\frac{1}{kh}\right)^2 + \frac{2}{3}(E_2E_0)^{1/2} \qquad (7)$$

The critical pressure for instability is different for each mode, and the first mode that becomes unstable when the osmotic pressure increases is given by

$$k_c = \frac{2\pi}{\lambda_c} = \left(\frac{E_0}{E_2}\right)^{1/4}\frac{\sqrt{3}}{h} \qquad (8)$$

and the critical osmotic pressure is

$$P_c = \tfrac{2}{3}(E_2E_0)^{1/2} \qquad (9)$$

The predictions that the wavelength of the pattern is proportional to the thickness of the gel, and that the critical pressure is independent of the gel thickness agree with the experimental results (see Fig. 3).

Once a mode becomes unstable and its amplitude increases, it is necessary to take into account the higher order terms.

$$V = \frac{K(k)}{2}\sum|A_{\mathbf{k}}|^2 - \frac{c}{2}\sum A_{\mathbf{k}_1}A_{\mathbf{k}_2}A_{\mathbf{k}_3} + \frac{d}{4}\sum|A_{\mathbf{k}_1}|^2|A_{\mathbf{k}_2}|^2 \qquad (10)$$

where the second summation is taken for $\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3 = \mathbf{0}$. This potential energy is minimized for a combination of three waves having wavevectors constituting an equilateral triangle relationship. Such a wave represents a hexagonal pattern (Fig. 4).

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Anomalous $^{234}U/^{238}U$ ratios in deep-sea hydrothermal deposits

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Although hydrothermal ferromanganese deposits often display variable $^{234}U/^{238}U$ ratios[1–5], published values generally do not differ by more than 20% from the seawater value of their hydrogenous counterparts[6]. Here we report very high $^{234}U/^{238}U$ ratios in hydrothermal manganese crusts recovered from the Sanghihe island arc system in the West Pacific. Such ratios, about twice that of normal seawater, confirm a low-temperature hydrothermal supply of uranium, which is best explained by leaching of the underlying rocks by the hydrothermal fluids and deposition together with the manganese oxides before mixing with seawater. This suggests that the $^{234}U/^{238}U$ in seafloor deposits may be used as a tracer of low-temperature hydrothermal reactions in the ocean crust.

During the past 10 years, major hydrothermal deposits have been discovered in association with both mid-ocean ridge systems and also island arc systems such as the South-West Pacific island arc (SWPIA)[7]. In the search for new hydrothermal sites associated with arc volcanoes, we attempted numerous dredges during the cruise 'ESTASE-2' of the RV *J. Charcot* on the flanks of several seamounts in the two arc systems of Sanghihe and Talau ridges adjacent to the Cotabato and Philippine Trenches, South Mindanao Island. U-series isotopes were shown to provide good tracers for the rock-weathering processes as well as dating tools for submarine deposits[8]. Here we report chemical and radiochemical data obtained on two dredge hauls from the western flank of a volcano that rises 2,300 m above a 5,000 m sea floor in the northern part of the Sanghihe Ridge (5°02′ N, 125°15′ E). Dredge DR4 (3,200-m depth) collected several large fragments of manganese crust 10–15 cm thick and DR11 (2,800 m depth) obtained similar Mn crusts, together with rocks coated by 1–2 mm of Fe–Mn oxides, pieces of manganese breccia and sediments.

The manganese deposits consist of alternating layers of compact and granular material similar to the crusts described by Moore and Vogt[4] in the Galapagos Spreading Center (GSC) and by Moorby *et al.*[9] in the SWPIA. Several samples contain lenticular areas of yellow to green or red smectite. Mineralogically, all the samples, which were not allowed to dry, contain a quite-well-crystallized todorokite in contrast to the hydrogenous incrustations that generally consist of δMnO_2. This todorokite is very unstable and heating results in a more or less complete conversion of the 10-Å to the 7-Å peak attributable to birnessite (Mellin *et al.*, in preparation). X-ray diffraction and chemical data on the smectite associated with the Mn oxides are very similar to those of the hydrothermal nontronite in the Galapagos Mounds[10].

Table 1 Chemical composition

Sample depth	Na (%)	Mg (%)	Al (%)	Si (%)	K (%)	Ca (%)	Mn (%)	Fe (%)	Ni (p.p.m.)	Co (p.p.m.)
DR4A/3 25–55 mm	3	1.1	<	0.5	0.9	1.5	47	0.022	30	7.7
DR4B/3										
11–23 mm	3.2	1.9	0.4	0.8	0.9	1.5	51	0.17	27	7.6
45–50 mm	3.1	1.7	0.5	0.9	0.9	1.3	51	0.02	21	7.5
DR4C/F 35–45 mm	1.7	1.6	0.3	1.3	1.1	1.3	50	0.78	20	12.5
DR11/E 10–20 mm	2.3	1.7	0.2	6.4	1.2	1.1	41.3	4.7	15	13
DR11/B 0.00–0.06 mm	2.1	1.3	2.0	7.3	0.5	1.8	12.9	18.5	4560	950
Sediment DR11	2.7	2	8.4	21	1.5	2.3	0.6	5.8	165	22
Nontronite DR11/A	1.2	2.1	0.7	21.4	2.1	0.5	2.3	19.1	<	6.5
Galapagos Mounds[5]	2.3			1.1			43.6		61	6.4
SWPIA hydroth. Mn crust[8]	2.9	1.1	0.08		0.55	1.3	47	0.02	14	7
Hydrogenous Mn endmember[11]	1.04	1.04	1.18	5.22	0.49	2.6	22.2	19.0	5500	1300
Suboxic diagenetic Mn end member[11]	3.28	1.38	0.75	1.63	0.62	1.25	48	0.49	4400	35

Table 2 Radiochemical data

Sample depth	U (p.p.m.)	Th (p.p.m.)	U/Th	^{234}U/^{238}U	^{230}Th (d.p.m. g^{-1})	^{227}Th as ^{231}Pa (d.p.m. g^{-1})	Age ^{230}Th/^{234}U Ky	Age ^{231}Pa/^{235}U Ky
DR4A/3 25–55 mm	4.29±0.16	<0.02	>200	1.50±0.04	2.39±0.10	0.102±0.016	72±6	59
DR4B/3 11–23 mm	3.60±0.07	0.12±0.04	30	1.45±0.08	2.19±0.08	0.096±0.020	86±6	75
45–50 mm	6.57±0.14	0.02±0.01	>200	1.44±0.02	3.81±0.11	0.176±0.019	81±4	76
DR4C/F 25–40 mm	3.47±0.17	0.07±0.02	49	1.87±0.03	2.089±0.005	0.076±0.006	59±2	51
DR11/E 10–20 mm	2.46±0.06	0.012±0.004	205	2.25±0.03	0.530±0.010	0.023±0.004	$14.8^{+0.8}_{-0.9}$	15.9
DR11/B 0.00–0.06 mm	11.3±0.5	35.1±2.4	0.32	1.18±0.04	270±10	91±4	excess	excess
Sediment DR11	0.95±0.05	2.3±0.2	0.41	1.06±0.09	3.22±0.20	0.450±0.050	excess	excess
Nontronite DR11/A	0.20±0.04	0.17±0.03	1.17	1.18±0.09	0.238±0.019	n.m.	excess	

n.m., Non-measurable.

The thin Fe–Mn coating of sample DR11/B consists of δMnO$_2$. This coating covers a piece of fresh dacite-andesite that was probably broken from the basement during dredging. The sediment itself, present in large amount in the dredge haul DR11 and adhering to the Mn crusts, consists mainly of detrital components: feldspar, quartz, kaolinite and montmorillonite.

Results of chemical analysis on the samples are given in Table 1; Na, Mg, Al, Si, K, Ca, Mn were obtained with an EDAX/SEM system, and Fe, Ni and Co were measured by INAA. The chemical composition of hydrothermal, hydrogenous and diagenetic manganese-rich deposits of deep-sea origin are also shown for comparison. The thin coating of DR11/B has the composition of typical hydrogenous deposits[11] with a Mn/Fe ratio of ~1 and a relatively high Co and Ni content. On the other hand, the chemical compositions of samples DR4 and DR11/E are similar to the hydrothermal crusts of GSC and SWPIA with high Mn (about 50%), low Fe and very low trace element (for example Ni and Co) content. These characteristics contrast with DR11/B and also with the diagenetic manganese deposit. Sample DR11/E resembles DR4 but has rather more Si and Fe, in the same ratio as in the nontronite; its composition may result from the mixing of the latter with the Mn-phase. The nontronite sample has very similar composition to that reported by Corliss *et al.*[10] in the Galapagos Mounds.

We have measured U and Th series isotopes in the four different phases present in the dredged samples: sediment, nontronite, manganese crust and the Fe–Mn coating. U and Th were measured by alpha-spectrometry by the same procedure as described in Reyss *et al.*[12]. For all the samples, the measurements were repeated without the ^{232}U spike to ensure the ^{234}U/^{238}U and the ^{227}Th/^{230}Th ratios were accurately established. For sample DR11/B, ^{210}Pb and ^{226}Ra were measured by gamma-spectrometry with a well-type Ge detector. Radiochemical data are presented in Table 2. The ages, deduced from ^{230}Th ingrowth towards radioactive equilibrium with ^{234}U and from ^{227}Th as ^{231}Pa ingrowth towards ^{235}U, are calculated on the assumption that the crust is a closed system and incorporated U free of Th and Pa at the time of formation. To lower the contamination by Th from hydrogenous or sedimentary components, we have selected samples with compact manganese phases represented by very low ^{232}Th contents. This sedimentary component, always important in the outermost layer, thus precludes the dating of the assumed youngest layer. Any contamination would lead to older ages and therefore the ages given are probably overestimated.

Sample DR11/B, identified as a hydrogenous incrustation from its chemical and mineralogical composition, has a high ^{232}Th content and the large excess of ^{230}Th and ^{231}Pa normally encountered in such deposits[6]. The ^{234}U/^{238}U ratio does not differ significantly from the seawater value of 1.14±0.03[13]. With a ^{210}Pb content of 770±70 d.p.m. g^{-1} and a ^{226}Ra content of 41±4 d.p.m. g^{-1}, we calculated a ^{210}Pb/^{226}Ra ratio of 19 and a ^{226}Ra/^{230}Th ratio of 0.15. This excess of ^{210}Pb and the deficiency of ^{226}Ra confirm that the crust was exposed to seawater at the time of sampling[14]. The nontronite sample has very low U and Th activities. This may result from contamination either by detrital sediment or by Mn as suggested by its Al and Mn content. Sampling of pure nontronite is impossible as it occurs only as thin lenses in the crusts, but our measurements confirm the strong fractionation of the elements during the hydrothermal process, with U being associated with the Mn oxides rather than with the Fe–Si precipitate.

The most striking feature in Table 2 is the high ^{234}U/^{238}U ratio measured in all the Mn rich crusts. The ratio of 2.25±0.03, about twice the seawater ratio, is the highest so far measured in an oceanic material. There are two indications that no post-depositional migration, as described in other deep sea deposits,[4,15] occurred in our samples: (1) in the crust DR4/B the U content of the deep layer (45–50 mm) is twice that of the shallower layer (11–23 mm) but the ^{234}U/^{238}U is identical, (2) the ages obtained from the ingrowth of ^{230}Th and ^{231}Pa to their parents ^{234}U and ^{235}U are in good agreement. Extensive mobility of the U isotopes within the crust that would change the ratio by a factor of two would lead to discordant ages by both methods.

The U isotopes found in the Mn layers have four possible sources: seawater, pore water, hydrothermal solutions and detrital sediments included in the oxides. As the U content of the crust is higher than that of the sediment, this last source can be safely rejected. There is no known mechanism by which the U isotopes of seawater could be fractionated during deposition; indeed the hydrogenous deposits display a typical seawater ^{234}U/^{238}U ratio, so that this source can also be discounted. All published radiochemical data on diagenetic Mn crusts[12,16-18] as well as direct measurements in pore waters[19] are close to the seawater ^{234}U/^{238}U ratio. This leaves a hydrothermal solution as the only possible U source with very high isotopic ratio.

In nature, U isotopes are fractionated by the effects of alpha recoil. Differences in bonding and oxidation state render the ^{234}U daughter isotope more mobile than its ^{238}U parent[8]. The anomalously high ^{234}U/^{238}U reported in hydrothermal Fe–Mn crusts[1-5] and in some late-stage volcanic products[20] have been attributed to such 'hot atom' effects. Krishnaswami and Turekian[21] and Michard *et al.*[22] measured U in hydrothermal vent solutions. They found no U in high-temperature fluids and deduced[21] that this element was deposited at temperatures ⩾30 °C. Following Rydell and Bonatti[2], they infer that seawater entering the system should be stripped of its U and deposited in hydrothermal conduits. Leaching of U from the rocks during shallow stages of sub-bottom circulation at lower temperatures may produce a solution relatively enriched in ^{234}U. The mixing of the hydrothermal solution with oxidizing seawater would result in variable ^{234}U/^{238}U ratios, ranging from the seawater ratio to higher values. In our samples, however, the ratios do not seem to be related to the ^{238}U content. Thus, a simple mixing process is not convincing. If the seawater U characteristics are known to be constant, the ^{234}U enrichment of the hydrothermal solution should depend on the residence time of the fluid in the rocks.

Kazachevskii *et al.*[23] measured a $^{234}U/^{238}U$ ratio of 1.14 ± 0.02 in Fe-Mn suspensions sampled directly on the flank of the volcano Banu Vukhu in the Sanghihe archipelago, the same arc system as our sampling area, where the emanation interacts with seawater. As the precipitation was observed to take place in seawater, it is likely that the hydrothermal U contribution in this sample was masked by the seawater imprint, as observed in other hydrothermal oxide deposits. On board, 3.5-kHz echo sounder records suggest that the crusts examined lay on a thin sedimentary cover. We hypothesize that the precipitation of the hydrothermal solution does take place in/at the top of this sediment before it is mixed with seawater. The origin of the U measured in the sediments from the Galapagos Mounds by Lalou and Brichet[24] may be due to a similar process as described by Moorby[25]. Several authors[26,27] have found evidence of a U source with $^{234}U/^{238}U > 1.14$, the extension of the present data to other low-temperature hydrothermal systems might clarify the hydrothermal solutions as the source involved. With the lack of direct measurements of the hydrothermal solution itself, we suggest that the anomalous $^{234}U/^{238}U$ ratio in seafloor deposits provide a probe for the low temperature hydrothermal reactions in the ocean crust and thereby could be used, together with ancilliary chemical information, to study chemical processes occurring in such systems. Work in progress in our laboratory is designed to contribute to this aim.

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Long-range transport of terrestrially derived lipids in aerosols from the south Pacific

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Terrestrially produced particulate organic matter can be transported relatively fast in the atmosphere, long distances over the ocean. It has been hypothesized that this atmospheric transport may be an important way of quickly introducing continentally-derived organic material to the surface waters of the open ocean[1–11]. After rapid transport through the water column to the sediment surface[12], these terrestrial organic substances could account for an important fraction of the organic carbon found in the sedimentary record[13]. The atmospheric fluxes of these organic substances are large enough to have a major potential impact on the inventory of terrestrially derived lipid material, originating from vascular plant waxes, found in deep-sea sediments[13]. We present here the first use of organic compound biological source marker information in conjunction with long-range meteorological trajectory analysis to elucidate specific terrestrial source regions and to determine the transport pathways of organic material through the atmosphere to remote marine locations.

The samples were collected as part of the south Pacific portion of the Sea/Air Exchange Program (SEAREX) in May–September 1983, from a 20-m tower erected on Ninety Mile Beach on the northwestern shore of the North Island of New Zealand. The site, 34.6° S, 172.8° W, is located in the Southern Hemisphere westerlies wind regime, approximately 1,500 km east of Australia. The sampling-analysis scheme for lipids in aerosols is summarized by Gagosian *et al.*[8] and the detailed analytical methods are presented in Peltzer *et al.*[14]. This sampling-analysis process requires stringent precautions against contamination during sampling, storage, transport, and analysis due to the extremely low concentrations of organic compounds found in remote marine air. The analysis involved extraction of glass fibre filter samples with methylene chloride, separation into lipid compound classes by silica-gel column chromatography and quantification by glass-capillary gas chromatography. Compound identifications were verified by gas-chromatography mass spectrometry and comparison of these spectra with authentic standards. Samples were accompanied by appropriate tower-to-analysis blanks. Recovery factors were determined by spiking the samples with appropriate internal standards[14].

To identify the sources of organic substances in aerosols, we have used the biomarker approach which takes advantage of the unique molecular signatures or source 'fingerprints' for various lipid compound classes of marine and terrestrial plants and animals and of anthropogenic activities[11]. Although these compounds make up approximately 10–50% of the solvent extractable organic matter in aerosols[8], they represent only a small portion of the total organic material. This 'tracer' approach for lipid class compounds is analogous to the use of natural and bomb produced radionuclides in atmospheric and oceanic source and transport studies.

Several conclusions concerning the sources of the organic material associated with aerosols can be made by examining the individual compound distributions within specific lipid compound classes. For all the samples analysed, the major source signature for the hydrocarbons was from terrestrial plant waxes[1,15,16] (Table 1, Fig. 1). For the *n*-alkanes, a strong odd-even carbon preference was observed, the major homologues being C_{29} and C_{31}. However, for the first time we also observed the lighter *n*-alkanes in the range C_{17}–C_{20}. This is most likely due to the lower ambient temperatures in New Zealand com-

Table 1 Atmospheric aerosol concentrations of aliphatic hydrocarbon, fatty alcohols, and fatty acid salts from samples collected from Ninety Mile Beach, New Zealand

Sample no. (mid-date/volume) (m^2)	NZAS-5 (19 June/4,000 m^3)	NZAS-16 (18 July/3,700 m^3)	NZAS-20 (12 Aug/4,400 m^3)	NZAS-22 (21 Aug/3,000 m^3)
Aliphatic hydrocarbons				
C_{17}–C_{40}*	22	61	186	270
CPI† (C_{17}–C_{40})	3.5	2.4	8.5	4.3
Major components†	1, 29, 33	29, 31, 27	31, 33, 29	31, 29, 33
tty alcohols				
$C_{13}C_{20}$*	16	10	16	14
Major components‡	16, 15, 17	20, 18, 15	20, 15, 18	20, 19, 18
C_{21}–C_{36}*	32	76	291	474
CPI† (C_{21}–C_{36})	9.2	14.4	13.3	22.1
Major components‡	26, 28, 32	26, 28, 30	26, 28, 32	28, 26, 32
Fatty acid salts				
C_{13}–C_{18}*	565	636	623	333
Major components‡	16, 14, 18	16, 14, 18	16, 14, 18	16, 18, 14
C_{19}–C_{36}*	65	86	81	98
CPI† (C_{19}–C_{36})	2.5	2.4	2.2	2.2
Major components‡	24, 20, 22	22, 24, 20	20, 24, 22	20, 22, 24

* Concentrations are in pg m^{-3} air.

† Carbon number in order of decreasing abundance.

‡ CPI, carbon preference index. Odd-even for aliphatic hydrocarbons, even-odd for fatty alcohols and acids.

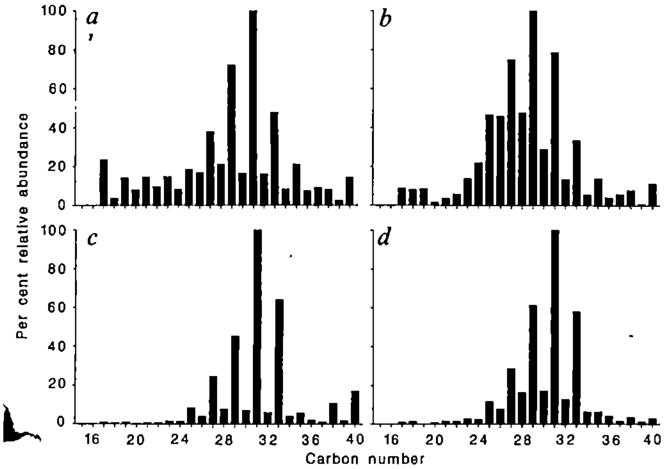

Fig. 1 Aliphatic hydrocarbon distribution patterns for aerosols collected at Ninety Mile Beach, New Zealand, June–August 1983. *a*, NZAS-5; *b*, NZAS-16; *c*, NZAS-20; *d*, NZAS-22. Total hydrocarbon concentrations are 22, 61, 186 and 270 pg m^{-3} respectively.

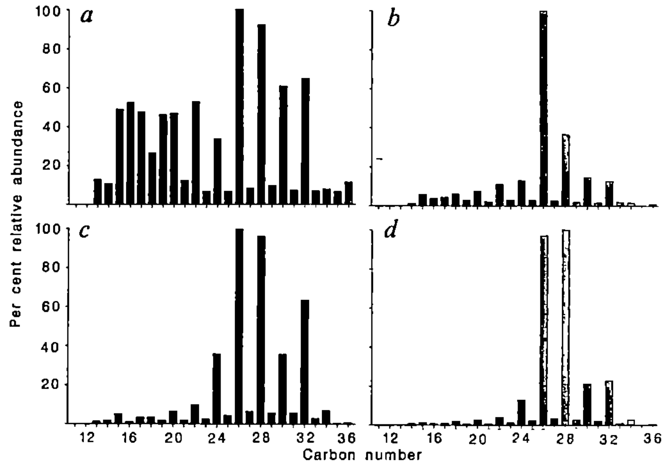

Fig. 2 Fatty alcohol distribution patterns for aerosols collected at Ninety Mile Beach, New Zealand, June–August 1983. *a*, NZAS-5; *b*, NZAS-16; *c*, NZAS-20; *d*, NZAS-22. Total fatty alcohol concentrations are 48, 86, 307 and 488 pg m^{-3} respectively.

pared to the higher tropical temperatures found at earlier sampling sites (American Samoa in the south Pacific[17] and Enewetak Atoll in the north Pacific[8]). For in-sector (ISS) westerly wind samples (NZAS-5, 20 and 22), which all had an Australian continental origin, C_{31} was the major homologue, where as C_{29} was the major component of the *n*-alkanes in aerosols coming from across New Zealand (NZAS-16). For many of the samples that we have analysed from American Samoa, C_{31} was the major *n*-alkane. This suggests that tropical *n*-alkane aerosol sources have higher carbon number homologues than their temperate counterparts[17]. The lower molecular weight source signature for NZAS-16 is consistent with its more temperate source.

The fatty alcohol distributions add further evidence to these source region assignments (Table 1, Fig. 2). In all the samples, a strong even-odd carbon preference for the C_{21}–C_{36} range was observed which is an indicator of terrestrial plant wax input[15,16]. Concentrations of the low molecular weight fatty alcohols (C_{14}–C_{20}) were low (10–20 pg m^{-3}) and fairly constant with time as expected for a marine source (Table 1). On the other hand, the terrestrially derived fatty alcohols (C_{21}–C_{36}) vary in concentration by over an order of magnitude. For the New Zealand ISS samples (NZAS-5, 20 and 22), the fatty alcohol mean carbon number was 27.5–28. At Enewetak the modal carbon number for the fatty alcohols showed a steady temporal progression from 27.1 to 29.3[13]. Based on long-range meteorological transport data[19], it was concluded that this progression was indicative of a shift from a temperate source in central Asia to a more tropical Central American source later in the year. In contrast to the three New Zealand ISS samples, the mean carbon number of the NZAS-16 fatty alcohols was 26.8. In addition, unlike most other samples where C_{26} and C_{28} are nearly equal in abundance, in NZAS-16 the C_{26} fatty alcohol was 42% of the total fatty alcohols and was three times more abundant than C_{28}. This indicates that NZAS-16 has a substantially different and more temperate source than the three ISS samples. Sample NZAS-15 which was taken one week before NZAS-16, has the same long range trajectory pattern and exhibits a similar fatty alcohol distribution, C_{26} being 58.5% of the total fatty alcohol concentration.

The low molecular weight (C_{13}–C_{18}) fatty acid salts appear

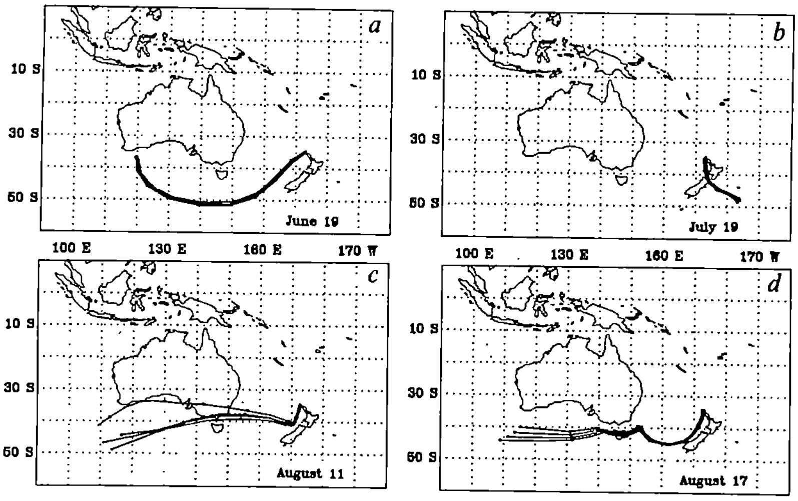

Fig. 3 Isentropic trajectories backward in time from the experiment site. Examples shown are at 290 K and are in the boundary layer at the site. They are representative of ensembles of trajectories calculated at surrounding points[19]. The dots on the trajectories are at one day intervals.

to have a marine origin[15]. In all of the New Zealand samples, C_{16} was the most abundant homologue followed by C_{14} and C_{18} (Table 1). This is comparable to the abundance patterns seen in the Enewetak[8] and Samoa[17] samples. The higher molecular weight (C_{19}–C_{32}) fatty acid salts in contrast show a pronounced land plant source as evidenced by the strong even-odd carbon predominance pattern and the lack of any real major homologue in the range C_{21}–C_{32}[15]. The mean carbon number of 24±0.5 is typical of this continental source as well.

Further information can be obtained concerning the source of the atmospheric aerosols by examining the New Zealand aerosol data in conjunction with the long-range transport and air parcel trajectory meteorological data. Figure 1 shows the aliphatic hydrocarbon concentrations for four New Zealand air samples. NZAS-5 had the lowest concentrations of all the New Zealand samples, 22 pg m^{-3} (Fig. 1*a*). The *n*-alkane composition for this sample had a lower mean carbon number than either NZAS-20 or NZAS-22 suggesting a more temperate source. The fatty alcohol composition of NZAS-5 (Fig. 2*a*) suggested a mixture of several sources including tropical, temperate and marine. Thus, based on chemical evidence we conclude that this sample represents southern ocean air with some minor continental influence.

NZAS-16 (Fig. 1*b*) had moderate levels of all three compound classes suggesting a stronger continental source than NZAS-5 but not nearly as strong as NZAS-20 or NZAS-22. The presence of anthropogenic *n*-alkanes in the hydrocarbon fraction (low CPI (carbon preference index), Table 1) is indicative of an urban source while the very low mean carbon number for the high molecular weight fatty alcohols (Fig. 2*b*) suggests that this sample had the most temperate source of all the samples analysed. Thus, we would predict that this sample represents an urban source in New Zealand. However, the *n*-alkane and terrestrial fatty alcohol concentrations were quite low (61 and 76 pg m^{-3} respectively) suggesting either that the transport was from high level winds over the source or that the source was quite distant.

Samples NZAS-20 and NZAS-22 had very high and similar concentrations of *n*-alkanes (186 and 270 pg m^{-3}) and terrestrial fatty alcohols (291 and 474 pg m^{-3}). The *n*-alkanes, the high molecular weight fatty alcohols and the high molecular weight fatty acid salts all indicate a major terrestrial source. Both samples exhibited a more tropical character than NZAS-16, NZAS-20 being the more tropical as exemplified by the high concentration of the C_{32} fatty alcohol. The levels of *n*-alkanes and fatty alcohols in NZAS-20 and -22 were greater than in any of our previous samples[8,11,17] suggesting a close continental source, most probably Australia.

In Fig. 3, we present airmass trajectories which are typical of the trajectories followed by the air samples in New Zealand for each of the aerosol samples. The trajectories were calculated using wind fields on isentropic surfaces found by the process described by Merrill *et al.*[18,19]. In each case, these trajectories are consistent with the predicted source of the aerosols based on the trace organic compound analyses. Figure 3*a* clearly shows a southern ocean origin for NZAS-5 which is essentially free of all continental influence for many thousands of miles. Some trajectories, during the period of sampling, approach the far southwestern part of the South Island of New Zealand. Most, however, are similar to the case shown, where the 'origin' (the position 10 days before arrival at the sampling site), is in the open ocean or near southwestern Australia. Figure 3*b* shows that NZAS-16 contains aerosols from New Zealand and that there was urban and terrestrial influence from the south of the North Island and Christchurch. The relatively low input of anthropogenic material found on the aerosol sample is supported by the trajectory analyses which show that the winds passed over New Zealand at heights above the boundary layer (800–900 mb). The trajectories for aerosol samples NZAS-20 (Fig. 3*c*) and NZAS-22 (Fig. 3*d*) originated in southern Australia and Tasmania and are consistent with the strong terrestrial biomarker signal. These examples of correlations of trace organic compound and air parcel trajectory analyses clearly show the benefits of this approach in defining aerosol sources and transport pathways. Future studies will help us further quantify source regions and place boundary conditions on the meteorological models.

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Primary production, new production and vertical flux in the eastern Pacific Ocean

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The sinking of particulate organic matter in the ocean links food webs beneath the euphotic zone to surface primary production and is an important pathway for the downward transport of many elements[1–3]. The flux of particulate organic carbon (POC) is also an important parameter in the global carbon cycle and may be related to long-term changes in atmospheric CO_2[4,5]. In 1980, Suess[6] synthesized existing measurements from sediment trap studies into a model to predict the vertical flux of POC from depth (z) and primary production (PP)[6]. The Suess model has become the standard for evaluating vertical flux data[7], for estimating the annual flux of POC in the ocean[8] and for parameterizing ocean carbon cycle models[4,5]. We present here a new model of the vertical flux of POC and particulate organic nitrogen (PON) from a set of contemporaneous measurements of PP and fluxes made during the VERTEX (Vertical Transport and Exchange) programme in the north-east Pacific. The VERTEX model indicates that PP and vertical fluxes of POC and PON, in the oligotrophic ocean are greater than previously suggested. In addition, the vertical flux of PON from the photic zone represents a measure of the PP that is supported by new nitrogen (new production)[9,10]. In the north-east Pacific, new production ranged from 13 to 25% of primary production and was positively related to total PP.

The vertical flux of POC and PON in the upper 2,000 m of the water column was measured using free floating particle traps[11] at nine sites in the Pacific Ocean[12,13]. While traps were deployed, PP was measured at each site, using the heavy-metal-free ^{14}C method (ref. 14). Trapped materials were preserved, in a formalin solution[15], as collected to avoid losses due to decomposition. Some organics undoubtedly partition from the particulate to dissolved fraction during preservation so the particulate fluxes measured in sediment traps represent conservative estimates[15,16]. All collections were also examined microscopically and zooplankton which had entered the traps alive during the deployment were removed before measurement of POC and PON[15].

The VERTEX data were $\log_{10}$-transformed and relationships for POC and PON were derived using multiple regression with z and PP as independent variables. We chose to express the data in terms of $mg\,m^{-2}\,d^{-1}$ instead of the units of $g\,m^{-2}\,yr^{-1}$ used in previous models to reflect more accurately the timescale of the measurements. The following equations for POC and PON were obtained:

$$\mathrm{POC} = 3.523\, z^{-0.734}\mathrm{PP}^{1.000} \qquad (1)$$

$$\mathrm{PON} = 0.432\, z^{-0.843}\mathrm{PP}^{1.123} \qquad (2)$$

where z is restricted to depths between the bottom of the euphotic zone and 2,000 m.

For comparison, we recalculated in terms of daily flux the original data from the model of Suess[6] and a reformulation of a limited portion of the same data by Betzer *et al.*[7] (Table 1). All models are highly significant; however, the amount of variance explained (r^2) in the VERTEX models is lower than previous models (Table 1). We attribute this partly to the higher variability that might be expected in contemporaneous measures of PP and flux relative to the averages of historical data used in previous models. In addition, increased flux rates with depth were occasionally observed, implying that horizontal advection of particles and *in situ* particle production may occur at specific depths[12,17,18].

The partial regression coefficients indicate the form of the relationship between vertical flux and the independent variables. For all three POC regressions, the partial regression coefficients for z are significantly < -1 (t-test, all $P < 0.001$). The nonlinear change in the relationship between flux and depth indicates, not unexpectedly[19], that most particulates are transformed to biomass, dissolved organics, and inorganic end products in the

Table 1 Summary of multiple regression models to predict the vertical flux of particulate organic carbon (POC) and nitrogen (PON) in mg C or N $m^{-2}\,d^{-1}$ from depth (z) in metres and primary production (PP) in mg C $m^{-2}\,d^{-1}$

Source	n	B_0	B_1	±95% CI	Range	$\bar{Z}$	B_2	±95% CI	Range	$\overline{PP}$	r^2	F	CF
Suess POC	33	−0.503	−0.567	0.104	50–5,582	780	1.302	0.164	110–1,644	345	0.97	507	1.066
Betzer *et al.* POC	27	−0.574	−0.627	0.121	100–5,582	1,392	1.415	0.151	110–1,644	272	0.97	433	1.043
VERTEX POC	74	0.547	−0.734	0.143	50–2,000	497	1.000	0.357	245–1,140	517	0.69	79.3	1.221
VERTEX PON	74	−0.364	−0.843	0.139	50–2,000	497	1.123	0.345	245–1,140	517	0.76	114	1.205

Data were $\log_{10}$ transformed and fitted to the equation $\log_{10}\mathrm{Flux} = B_0 + B_1\log_{10}z + B_2\log_{10}\mathrm{PP}$. Additional statistics are the means and ranges of the data used in each model as well as the coefficient of determination (r^2) and F-value from the multiple regression analysis of variance (for all 4 models $P < 0.0001$). Predictions in arithmetic units from these logarithmic regressions must be corrected for bias introduced in back transformation using the calculated correction factor (CF)[27].

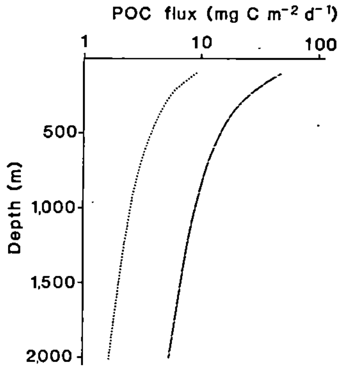

Fig. 1 Comparison of the Suess (dotted line) and VERTEX (dashed line) models at primary productivities representative of oligotrophic oceanic conditions. For the Suess model a historical average PP of 100 mg C m^{-2} d^{-1} was used. For the VERTEX model the median measured oceanic PP of 400 mg C m^{-2} d^{-1} was used.

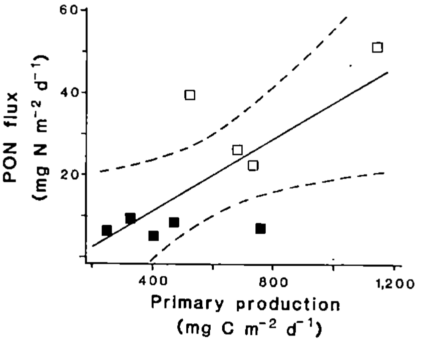

Fig. 2 Relationship between primary production and vertical flux of particulate organic nitrogen (PON) measured in traps located at the base of the euphotic zone for nine VERTEX stations. Regression model: PON $= 0.0439$ PP $- 6.17$, $r^2 = 0.52$, $F = 7.57$, $P < 0.01$. Solid squares represent stations with deep euphotic zones (≥ 100 m) and open squares represent stations with euphotic zones of 50 m. Broken lines are the 95% confidence intervals for the regression.

upper water column. The partial regression coefficients for PP are typically >1, but not significantly so for the VERTEX models. The hypothesis that the ratio of flux to production increases with PP ($B_2 > 1$) (see ref. 7) is not supported by the VERTEX data.

The PP data used in the Suess model reflect historical measurements and are lower than measurements made in the VERTEX programme. For example, daily productivities of the order of 100–150 mg C m^{-2} d^{-1} were considered representative of oligotrophic oceanic conditions[6]. Measurements of PP made at 5 oligotrophic sites during VERTEX ranged from 245–760 mg C m^{-2} d^{-1} with a median value of 400 mg C m^{-2} d^{-1}. Figure 1 compares predicted POC fluxes for the Suess and VERTEX models at PP of 100 and 400 mg C m^{-2} d^{-1} respectively. The VERTEX model POC fluxes are 3–5 times greater (depending on depth) under these conditions. The actual rates of PP in the open ocean have recently been intensely debated[20–25]. The VERTEX data, although limited, support the notion that oceanic PP is higher than historical estimates and that the vertical flux of sinking particles is also greater.

The flux of PON from the euphotic zone represents a measure of new production, since this flux must ultimately be balanced by inputs of nitrogen to the euphotic zone[9,10]. For the nine study sites, the relationship between PP and the flux of PON is significant (Fig. 2). Using the linear regression model of Fig. 2 and assuming a C/N ratio of 6.6, new production as a percentage of the total increases from 13 to 25% over the PP range of 245–1,140 mg C m^{-2} d^{-1}. Confidence, however, in any prediction of new production from measures of PP and our model is poor (Fig. 2). This might be interpreted as support for the notion of high variability in new production[24], but for most oligotrophic sites studied with euphotic zone depths ≥ 100 m ($n = 5$, solid squares, Fig 2), new production as measured by PON flux was relatively constant: $\bar{x} \pm 1$ s.d. $= 7.23 \pm 1.59$ mg N m^{-2} d^{-1} (Fig. 2). If we convert the daily mean new production value for oligotrophic conditions to an annual rate by multiplying by 365 and assuming a Redfield C/N ratio of 6.6, the estimate of 17.4 ± 3.83 g C m^{-2} yr^{-1} is reasonably close to the value of 25.2 ± 6.96 g C m^{-2} yr^{-1} recently estimated for the Sargasso Sea[23]. Our value, however, is significantly less than the 50 g C m^{-2} yr^{-1} estimated by Jenkins and Goldman[23]. The data are clearly too limited to provide an accurate estimate of new production, given the potential spatial and temporal variability. A VERTEX seasonal study to measure the variability in PP and PON flux over an annual cycle at an oceanic site is now underway.

The empirical models of vertical flux allow prediction of the rate of loss of organic matter from the photic zone, the transformation of this material in the mesopelagic zone, and input to the deep sea. We conclude that the magnitude of vertical flux in the ocean may be greater than previous models based on PP suggest, because oceanic PP may be higher than historical averages. The coupling of models of vertical flux as developed in the VERTEX study with improved models of circulation and PP based on remote sensing will substantially enhance our knowledge of the flux and cycling of materials on seasonal, regional and basin-wide scales in the ocean[26].

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Molecular genetic evidence for heterogeneity in manic depression

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Manic depression is a severe cyclic mental illness[1,2] that can be unipolar or bipolar and has a lifetime risk of approximately 7 per 1,000 in most populations[3]. Families with multiple cases of manic depression have been described that are compatible with both autosomal dominant and X-linked modes of genetic transmission[2,4–6]. Psychoactive antidepressant and stimulant drugs that help to ameliorate depression and mania are thought to act by affecting catecholamine neurotransmitter systems such as adrenaline, noradrenaline and dopamine, amongst others[7]. Mutations affecting the tyrosine hydroxylase (*TH*) gene[8,9], which encodes the rate-limiting enzyme for the synthesis of these three neurotransmitters[7], might therefore be responsible for causing the manic depressive phenotype. We have studied three Icelandic kindreds amongst whom it appears that a single autosomal dominant disease allele is segregating. In these families there were 44 cases amongst 73 individuals at risk. Genetic linkage studies were carried out using clones encoding tyrosine hydroxylase[8,9] the variable portion of the Harvey-*ras*-1 (*HRAS1*)[10] locus and the variable region of the insulin gene (*INS*)[11]. All three markers are closely linked on chromosome 11 (ref. 12) and were used to observe the segregation of restriction fragment length polymorphisms (RFLPs) in the three affected kindreds. We found no evidence for linkage to these markers in any of the three families. In contrast, Gerhard *et al.*[13–15] found linkage between manic depression and *HRAS1* in a single large Amish kindred. We conclude that there is genetic heterogeneity of linkage in manic depression. Therefore mutations at different loci are responsible for the manic depressive phentoype in the Amish and in Iceland.

The discovery of polymorphic DNA markers has made it possible to begin a systematic search using linkage methods for mutations causing manic depression. However, complicating factors such as heterogeneity of linkage, and incomplete and age-related penetrance need to be overcome. We therefore identified large multiply affected families for whom it was very likely that a single genetic mutation had been responsible for the manic depressive phenotype.

An important issue for psychiatric genetic investigations is the definition of the phenotype to be studied. We employed the widely used Research Diagnostic Criteria (RDC)[16] to establish psychiatric diagnoses. Information required to reach diagnoses was obtained by a structured interview carried out by a psychiatrist with the Lifetime Version of the Schizophrenia and Affective Disorders Schedule (SADS-L)[16] as well as by review of case notes. Summaries of all the clinical histories were compiled and the SADS-L RDC diagnoses were confirmed by two additional psychiatrists. Three Icelandic kindreds (F2, F6, F13) were identified and extensive psychiatric information on past generations was obtained in order to establish firmly that there was a single unilateral source of manic depression. One of the pedigrees (F2) is shown in Fig. 1.

Table 1 Lod scores between *HRAS*1 and manic depression at various values of recombination fraction (θ)

	Recombination fraction (θ)						
	0.00	0.001	0.05	0.10	0.20	0.30	0.40
Penetrance							
Model I	$-\infty$	−18.01	−5.10	−3.07	−1.16	−0.28	+0.06
Model II	$-\infty$	−16.78	−4.99	−2.99	−1.11	−0.24	+0.08
Model III	$-\infty$	−10.02	−3.24	−1.98	−0.72	−0.11	+0.10
Model IV	$-\infty$	−12.31	−4.41	−2.72	−1.04	−1.04	+0.06

In two of the three families, six of the cases were unipolar (UP) depressives who were also alcoholic. Because there is a complex interaction between alcoholism and depression[25], we excluded, on an *a priori* basis, all cases of alcoholism for the purposes of linkage analysis. After these exclusions, there were 38 cases out of 62 individuals at risk, of these 20 were bipolar (BP) cases and 18 were UP. This proportion is not significantly different from that expected under an autosomal dominant model. Allele frequences for *HRAS1* were based on data from White *et al.*[11]. Gene frequency for manic depression in the Icelandic population was estimated to be 0.01[26]. To test the effect of various penetrance functions on the outcome of the study, two-point lod scores were computed using four different penetrance models. In each model penetrance was assumed to be a function of age and an age-of-onset curve was constructed. In model I, the age-of-onset curve was obtained for each family individually by dividing the total number of affected cases by the number who were affected before the ages of 19, 29, 39, 49, 59 and 79 years. Under this assumption penetrance values for the six age groups were, for pedigree F2, 0.43, 0.86, 0.86, 1.00, 1.00, 1.00; for pedigree F6, 0.06, 0.45, 0.8, 1.00, 1.00, 1.00; for pedigree F13: 0.16, 0.76, 0.92, 1.00, 1.00, 1.00. In model II, penetrances were calculated for all three families combined: 0.14, 0.51, 0.70, 0.89, 1.00, 1.00. In model III, penetrances were set equal to those found in the Amish study: for ages 15–19, 0.10; 20–24, 0.27; 25–29, 0.56, and 30+, 0.63. Two-point lod scores were also calculated for complete penetrance (model IV).

Genomic DNA was digested with *Hin*fI, *Bgl*II and *Pvu*II restriction enzymes (Amersham) and separated by electrophoresis on agarose gels[17]. DNA was transferred by Southern blotting[18] to nylon filters (Hybond N). Pedigree structure was confirmed by hybridization with mini-satellite[19,20] probe 33.15. Four chromosome 11 linkage markers were investigated in the families. Erythrocyte MER2 antigen, encoded by a gene closely linked to *TH* on chromosome 11[21], was not found to be informative. A 1.5-kilobase (kb) fragment (Ty7) of a *TH* cDNA clone was used to detect a *Bgl*II dimorphism at the *TH* locus[12]. The

Table 2 Lod scores for linkage between three marker loci and manic depression for various values of recombination fraction (θ), using model II

	Recombination fraction (θ)						
	0.00	0.001	0.05	0.10	0.20	0.30	0.40
HRAS1							
F2	$-\infty$	−11.39	−3.05	−1.71	−0.56	−0.09	+0.07
F6	$-\infty$	−2.36	−0.66	−0.36	−0.09	+0.03	+0.05
F13	$-\infty$	−3.03	−1.28	−0.92	−0.46	−0.18	−0.04
INS							
F2	—	—	—	—	—	—	—*
F6	$-\infty$	−9.85	−1.68	−0.54	+0.16	+0.18	+0.03
F13	$-\infty$	−11.09	−2.79	−1.50	−0.45	−0.07	+0.01
TH							
F2	−0.05	0.05	0.04	0.00	0.08	0.12	0.09
F6	$-\infty$	−8.39	−1.81	−0.86	−0.21	+0.02	+0.01
F13	$-\infty$	−4.80	−1.44	−0.89	−0.39	−0.15	−0.03

Scores for each family are given separately. An age-of-onset curve was used on the combined penetrance estimates for all 3 families (Model II, as described in Table 1).

* Data not available for F2.

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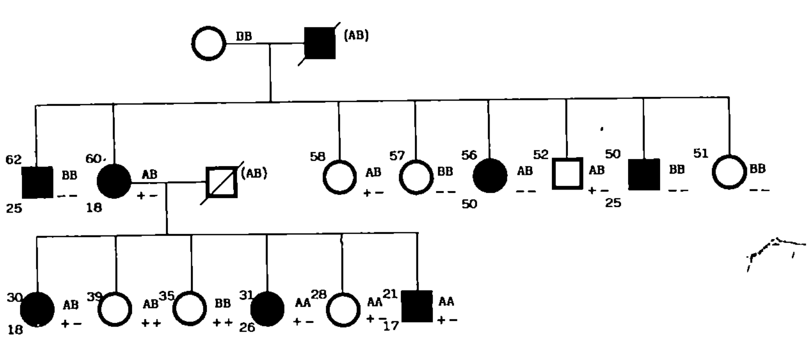

Fig. 1 F2 pedigree structure showing age (above) and age of onset (below) for each individual, *HRAS1* (A, B) and *TH* (+, −) alleles. Squares males; circles, females. Filled symbol, bipolar case; half-filled symbol, unipolar case; open symbol, unaffected; diagonal line, decreased.

variable tandem repeat (VTR) of *HRAS1*[10] was examined in *Hin*fI-digested genomic DNA using a 1.4-kb *Ava*II fragment probe containing the VTR 3′ to the *HRAS1* locus and four alleles were observed. In two families, F6 and F13, an 879-base-pair (bp) *Pvu*II fragment containing the 5′ hypervariable region flanking the insulin (*INS*) gene[11,22] was used to detect 5 different alleles in *Pvu*II-digested genomic DNA. The *HRAS*1 and *TH* genotypes in F2 are shown in Fig. 1.

The computer package LINKAGE[23] was used to calculate lod scores between disease and marker loci (lod scores quantify the likelihood of linkage between these loci) under different penetrance models. Table 1 gives lod scores for linkage between *HRAS1* and manic depression summed for all families for each model of penetrance. These data exclude the possibility of close linkage between manic depression, *TH*, *INS* and *HRAS1* provided that there is no genetic heterogeneity between families. To assess this possibility, two-point lod scores for the three markers were calculated for each family separately (Table 2). These data provide no evidence for linkage between the disease locus and any of the markers in any of the three families studied. The effect of the exclusion of alcoholics on the linkage analysis was assessed by computing lod scores with all members of the pedigree included. No evidence for linkage was found (data not shown). The three marker loci are closely linked[11,12], so multipoint analysis was carried out using the program LINKMAP so the data contributed by each locus could be combined. Linkage between manic depression and the marker loci was excluded ($Z < -2.00$) for a region of ~20 centimorgans on both sides of *HRAS1*.

We conclude that mutations at different loci are causing manic depression in the Old Order Amish and in Iceland. Synthetic gene frequency maps show that the Icelandic population shares the greatest genetic similarity with the populations of northern Germany and with other northern Europeans[24]: the Amish are known to have migrated from Germany. Therefore further research is required to establish the degree of genetic heterogeneity of linkage that is involved in manic depression.

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Close linkage of c-Harvey-*ras*-1 and the insulin gene to affective disorder is ruled out in three North American pedigrees

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Affective disorder (AD) is one of the major forms of functional psychoses. Although the mode of transmission is uncertain, family, twin and adoption studies strongly suggest a genetic involvement (see ref. 1 for review). Because a basic biochemical abnormality is not known, direct analysis of the disease using a probe for the defective gene is not possible. However, a specific locus can be tested for its relevance to the aetiology of AD by genetic linkage, using restriction fragment length polymorphisms (RFLPs). Using probes for the c-Ha-*ras*-1 oncogene and the insulin gene, Gerhard *et al.*[2] and Egeland *et al.*[3] found convincing evidence for close linkage between these markers and a locus for AD in a large Old Order Amish pedigree. In an attempt to confirm this finding, we examined three bipolar pedigrees outside the Amish population. Our results indicate the absence of linkage from 0 to 15% recombination frequency between AD and the insulin gene–*HRAS1* region in these pedigrees.

Pedigrees in which several members have major affective disorders were recruited through bipolar patients at the National

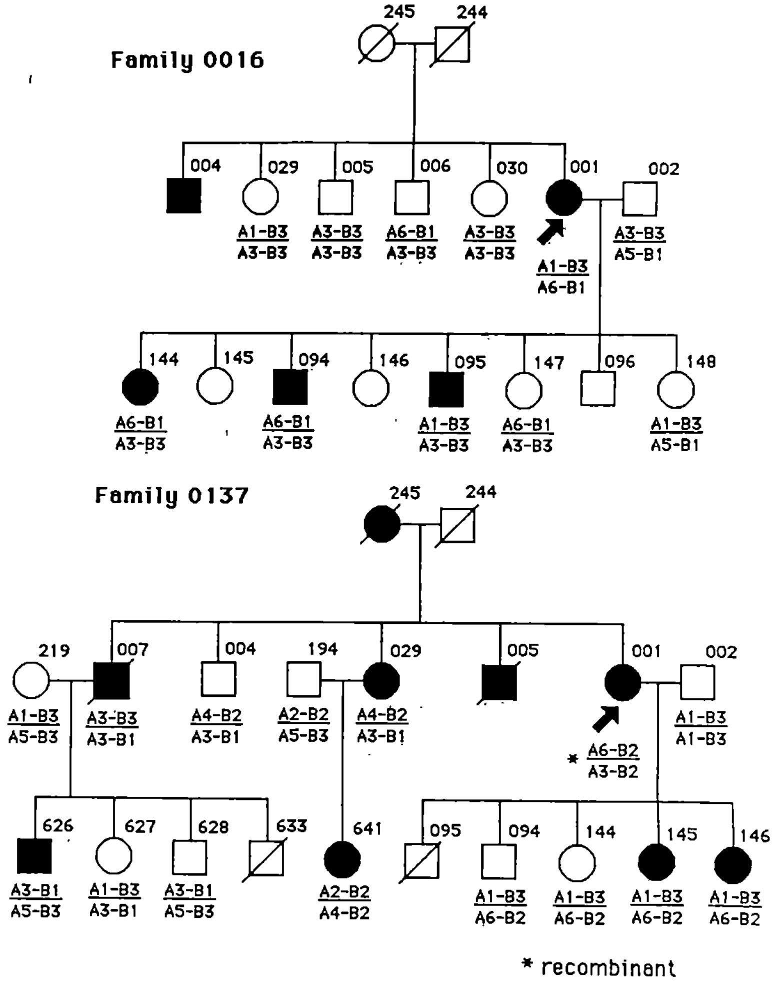

Fig. 1 Segregation of the insulin gene and *HRAS1* haplotypes in two pedigrees with major affective disorder. *A*1-6 and *B*1-3 are the haplotypes of the insulin gene and *HRAS1* (see text). Affected members are denoted by solid symbols; circles, females; squares, males; diagonal line, deceased; arrow, propositions. Genomic DNAs from indicated individuals were prepared either directly from peripheral blood or from cultured lymphoblasts transformed by the Coriell Institute for Medical Research, Human Genetic Mutant Cell Repository, Camden, New Jersey, USA. One set of aliquots of DNA was digested with *Pvu*II and another with *Sac*I. The DNA fragments were electrophoresed on agarose and transferred to nylon membranes (ICN) by the method of Southern[20]. The *Pvu*II blots were hybridized with the ^{32}P-labelled insert from phins 310 (a generous gift of Dr Graeme Bell, Chiron Corporation) and the *Sac*I blots were hybridized with ^{32}P-labelled EJ6.6[12] insert (American Type Culture Collection). The DNAs were labelled according to Feinberg and Vogelstein[21,22] to a specific activity of 10^9 c.p.m. μg^{-1}. Hybridization was performed for 48 h at 42 °C with 50% formamide, 5×SSC, 2×Denhardt's, 20 mM Na$^+$ phosphate, *p*H 6.5, 200 μg ml^{-1} denatured salmon sperm DNA, 0.1% SDS and 10% dextran sulphate and denatured ^{32}P-labelled probe. The filters were washed at room temperature with 2×SSC, 0.1% SDS three times, 15 min each time, followed by at least one wash at 65 °C for 1 h. Autoradiography was done using Kodak XAR-5 films with Dupont Lightning Plus intensifying screens at −70 °C for at least 2 days.

Institute of Mental Health. Diagnoses were determined using modified Research Diagnostic Criteria based on the Schedule for Affective Disorders and Schizophrenia Lifetime version[4] interview, information from relatives, and review of all available hospital records[5]. In the present study individuals with a diagnosis of bipolar I (BPI) are classified as 'affected'. Also included in this category are bipolar II (BPII) with major depression and unipolar (UP), based on family[1] and twin studies which find these diagnoses greatly increased in relatives of manic depressives as compared with relatives of normal controls[1,5]. Schizoaffective and anorexic relatives are classified as affected because of the high prevalence of BP and UP disorders in families of these patients[5,6]. Family members with minor depression alone were classified as 'unaffected'[5]. The pedigrees collected were North American caucasians of European origin.

Attempts to correlate specific loci with AD have yielded primarily negative results[7,8]. The *HLA* region was initially believed to be loosely linked to AD but later studies by other investigators failed to confirm this finding (see ref. 7 for review). Other studies have suggested X-linkage in a proportion of families with AD, but the data have not been consistently reproducible[7]. We have obtained evidence suggesting that somatostatin and neuropeptide Y RFLPs are not linked to an AD locus (unpublished results). Proopiomelanocortin RFLPs were found not to be associated with bipolar illness[8].

Recent data obtained from the study of an Amish pedigree demonstrate linkage of BP and UP affective disorders to the insulin gene-*HRAS1* region of chromosome 11[3]. The multiple alleles of the insulin and *HRAS1* genes[9-11] make this region of chromosome 11 extremely useful for genetic linkage study. The high degree of polymorphism at the 5′ flanking region of the insulin gene results from the variation in the number and arrangement of tandemly repeated nucleotides[10]. When *Pvu*II is used, up to six different restriction fragments can be detected using phins 310 as probe and these alleles are designated A1 to A6[9-11].

The oncogene *HRAS1*, in close proximity to the insulin gene, has been shown to be highly polymorphic (ref. 9 and D. S. Gerhard, personal communication). Multiple polymorphic fragments, designated here as B alleles, have been observed when *Sac*I is used as the enzyme and E.J.6.6 as probe (ref. 12 and D. S. Gerhard, personal communication).

Two of the three pedigrees examined for insulin and *HRAS1* RFLPs are shown in Fig. 1. The total number of individuals examined in all three pedigrees was 38, 15 of whom were affected. As indicated, haplotypes were deduced from the genotypes obtained with the insulin gene and *HRAS1*. This was done because the genetic distance between these genes is only 3%[13]. In the three pedigrees studied only one recombinant was found, individual 137001 (Fig. 1). She was assigned a haplotype according to her *HRAS1* genotype because the results from the Amish study[2,3] indicate close linkage of AD to the *HRAS1* gene. The corresponding age and diagnosis of each family member studied are summarized in Table 1.

Linkage tests between marker haplotypes and affective illness were performed by estimation of lod scores using the LIPED[14] program. A correction for age was incorporated in the calculations. For example, the normal diagnosis for individuals 16147 and 16148 could change with time as they are just approaching the age of risk. Population prevalence also enters into lod score computations, in assigning a gene frequency for the disease allele. For BP illness and acute schizoaffective disorder, the combined population prevalence is ~1%[5]. For UP illness, varying rates have been reported which are a function of the diagnostic criteria used, birth cohort and other factors[15,16]. With stringent criteria, we have estimated the population rate to be 5.8%[5].

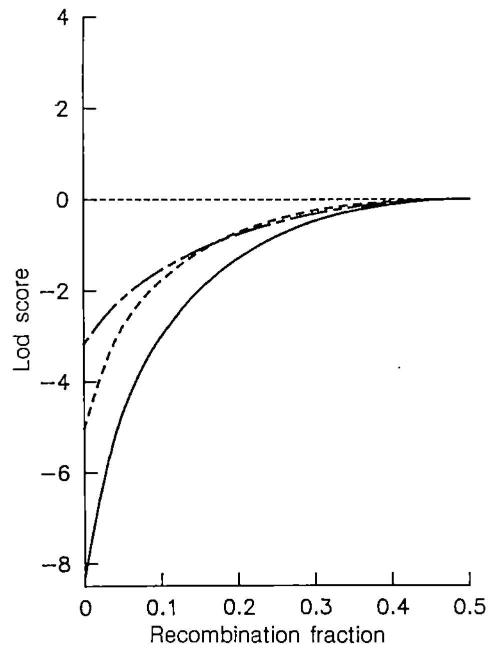

Fig. 2 Graph of the combined lod scores from 3 pedigrees as a function of recombination fraction to determine linkage between an AD locus and the insulin gene–*HRAS1* region. The lod scores were calculated based on haplotypes determined from insulin and *HRAS1* genotypes as shown in Fig. 1. The computer program LIPED[14,23] was used under three models with a straight-line age correction (age of risk specified as 12 to 65 years). These models were: (1) dominant with reduced penetrance (long and short dashed line), where $q_a = 0.035$, $f_{aa} = f_{Aa} = 0.5$, $f_{AA} = 0.005$; (2) recessive (short dashed line), where $q_a = 0.20$, $f_{aa} = 0.9$, $f_{Aa} = f_{AA} = 0.005$ and; (3) dominant model (solid line) using assumptions from the Amish pedigree where strong evidence for linkage was found[2,3], that is, $q_a = 0.015$, $f_{aa} = f_{Aa} = 0.85$, $f_{AA} = 0.001$, age of risk specified as 15 to 45 years. Genotype penetrances are denoted by f_{aa} for abnormal homozygote, f_{Aa} for the heterozygote and f_{AA} for the normal homozygote: q_a is the frequency of the recessive allele. Linkage is rejected at the recombination fractions with lod scores less than −2.0 (ref. 19). Close linkage is rejected for all models tested.

Table 1 Diagnoses and ages of pedigree members

Individual no.	Age	Pedigree 16 Diagnosis
001	54	BPI
002	52	unaffected
005	59	unaffected
006	57	unaffected (minor depression)
029	61	unaffected
030	51	unaffected
094	28	BPI, drug abuse
095	24	BPI
144	32	BPI
147	23	unaffected
148	21	unaffected

Individual no.	Age	Pedigree 137 Diagnosis
001	65	BPII
002	64	unaffected
004	73	unaffected
007	68	BPI
029	70	BPI
094	38	unaffected
144	34	unaffected
145	28	BPII, major depression, anti-social, bulimia, drug abuse, alcoholism
146	26	minor depression, anorexia, bulimia
194	65	unaffected (alcoholism)
219	65	unaffected (minor depression)
626	30	probable UP
627	33	unaffected
628	34	unaffected (panic disorder)
641	32	schizoaffective, manic, depressed

The family members included here are only those for which DNAs were available. The ages shown were either at last examination or death.

Similar rates have been obtained in other independent studies[17,18]. Up to four times higher rates have been reported using more inclusive criteria[15]. For the linkage analyses presented here, we assume that these 'high-density' families represent a rarer subtype of AD, and have based our genetic models on a prevalence rate of 3.5%, which is half our estimated combined prevalence rate for BP, schizoaffective and UP disorders. However, the lod scores are not substantially changed within a considerable range of gene frequencies (data not shown).

To compare our results with those obtained by Gerhard *et al.*[2] and Egeland *et al.*[3], a dominant mode of inheritance with 85% penetrance was assumed. At 0–15% recombination the combined lod scores obtained from the three pedigrees examined in the present study were less than −2, which allows rejection of close linkage[19]. The results are graphically presented in Fig. 2. A similar observation was seen when pedigree 137 was examined alone (data not shown). Negative lod scores were also obtained when two other models, dominant with reduced penetrance and recessive, were tested (Fig. 2). When the insulin and *HRAS1* loci were analysed separately in these pedigrees, assuming in each case the mode of inheritance used in the Amish study[2,3], linkage to AD is rejected at less than 5% recombination (data not shown). When the haplotype of individual 137001 was assigned according to her insulin genotype the change in the lod score values was not significant (data not shown). If we narrow our classification of affective diagnoses to include only BP, UP and schizoaffective or only BP and schizoaffective the lod scores are slightly higher but our conclusions are not changed (data not shown). Our data, therefore, support the hypothesis of independent segregation of the insulin gene–*HRAS1* region and affective illness. This contrasts with the evidence reported earlier[2,3]. The reason for this discrepancy is not clear. Conceivably these contrasting findings favour aetiological heterogeneity in AD. The AD in the Amish pedigree may represent a genetically distinct form of the illness. However, the hypothesis of genetic heterogeneity will remain speculative until additional pedigrees and more regions of the genome are studied.

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Cholecystokinin induces a decrease in Ca^{2+} current in snail neurons that appears to be mediated by protein kinase C

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Three distinct classes of protein kinases have been shown to regulate Ca^{2+} current in excitable tissues. Cyclic AMP-dependent protein kinase mediates the action of noradrenaline on the Ca^{2+} current of cardiac muscle cells[1,2]. Cyclic GMP-dependent protein kinase mediates the serotonin-induced modulation of the Ca^{2+} current in identified snail neurons[3]. The Ca^{2+}/diacylglycerol-dependent protein kinase (protein kinase C) has also been found to regulate Ca^{2+} currents of neurons[4,5]. However, no neurotransmitter has yet been shown to regulate Ca^{2+} current through the activation of protein kinase C. We now report that cholecystokinin, a widely occurring neuropeptide[6], which is present in molluscan neurons[7,8], modulates the Ca^{2+} current in identified neurons[9] of the snail *Helix aspersa*, and that this effect appears to be mediated by protein kinase C. Specifically, sulphated cholecystokinin octapeptide 26–33 (CCK8), activators of protein kinase C, and intracellular injection of protein kinase C, all shorten the Ca^{2+}-dependent action potential and decrease the amplitude of the Ca^{2+} current in these cells. All these effects are not reversible within the duration of the experiments. Moreover, intracellular injections of low concentrations of protein kinase C, which are ineffective by themselves, enhance the effectiveness of low concentrations of CCK8 on the Ca^{2+} current.

The effect of CCK8 on the somatic Ca^{2+}-dependent action potential was measured in the identified snail neurons F77 (Fig. 1*a*) and D2 (not shown) bathed in tetraethylammonium (TEA)/Cs^+-saline. CCK8 (5 nM) reduced the duration of the Ca^{2+}-dependent action potential, a maximum decrease being measured 7 min after bath application of the peptide. The effect of CCK8 was not reversed by washing the preparation for 45 min (not shown).

We next analysed the effect of CCK8 on the ionic currents in these cells. The effect of CCK8 on the inward current was measured in F77 and D2 neurons bathed in tetrodotoxin (TTX)/TEA/Cs^+/Ba^{2+}-saline. CCK8 (5 nM) decreased the inward current amplitude, a stable 20–25% decrease being obtained 7 min after the peptide application (Fig. 1*b*). No recovery occurred even after washout of the peptide for 40 min. The threshold concentration of CCK8 for an effect on the inward current was 0.5 nM, and a maximal effect (reduction of 70–80%) was obtained after 1–2 min with 500 nM CCK8. The *I*-*V* curves shown in Fig. 1*c* confirm that CCK8 decreases the inward current through Ca^{2+}-channels. CCK8 (5 nM and 20 nM) decreased the inward current at all membrane potential levels examined. The membrane potential level at which the inward current was maximal was the same in control and treated neurons, and at this potential value, CCK8 induced a maximal decrease in inward current. CCK8 action on the inward current through Ca^{2+}-channels was confirmed using the ionophore nystatin to exchange intracellular K^+ for Cs^+ (see ref. 10), and the extracellular Ca^{2+} was replaced by Ba^{2+} and NaCl by TrisCl. Under these conditions, in which Cs^+ was the only intracellular monovalent cation and K^+ and Na^+ were absent from the extracellular medium, the effect of CCK8 on the amplitude of the inward current was unchanged. We also tested the effect of the peptide on the TEA-insensitive, Ca^{2+}-independent outward current in F77 and D2 neurons bathed in TEA/Cs^+-saline in which Ca^{2+} was replaced by Mg^{2+}. In these experiments ($n = 4$), the cells were held at −50 mV and depolarized to +20 mV for 1 s. The application of CCK8 (5 nM) had no effect on the outward current in any of these experiments (not shown).

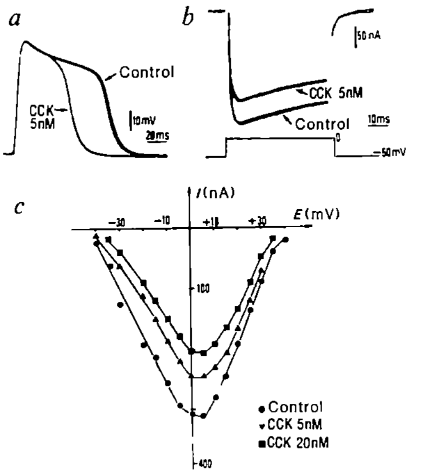

Fig. 1 Effect of CCK8 on the Ca^{2+}-dependent action potential and the inward current recorded in identified snail neurons. *a*, A control action potential showing a large Ca^{2+}-dependent plateau, was recorded from a F77 neuron bathed in TEA/Cs^+-saline. Exposure to 5 nM CCK8 elicited a decrease in the duration of the action potential. Scale bars are 10 mV (vertical) and 20 ms (horizontal). *b*, A control inward current was recorded from a different F77 neuron bathed in TTX/TEA/Cs^+/Ba^{2+}-saline. Exposure to 5 nM CCK8 elicited a decrease in the peak amplitude of the inward current. A constant value was reached after 7 min. Scale bars are 50 nA and 10 ms. *c*, *I*-*V* curves relating the inward current peak amplitudes and the membrane potential at which they were recorded in a D2 neuron bathed in TTX/TEA/Cs^+/Ba^{2+}-saline. The values of the peak inward currents were obtained in the absence (circles) or presence of 5 nM (triangles) and 20 nM (squares) CCK8. The three curves were corrected for linear leakage conductance.

Methods. The isolated and desheathed abdomino-visceral ganglionic mass of the snail *Helix aspersa* was bathed in saline of the following composition (mM): NaCl 120, KCl 2.5, $CaCl_2$ 6, $MgCl_2$ 3.5, HEPES 10 (*p*H 7.4). Identified snail neurons (D2 and F77, ref. 9) were impaled with two microelectrodes connected to a current/voltage clamp system. To unmask the Ca^{2+}-dependent component of the action potential, some of the K^+-conductances were blocked by replacing one-fourth of the NaCl with 30 mM TEA Cl and replacing KCl by CsCl in the saline (TEA/Cs^+-saline). To isolate the inward current through Ca^{2+}-channels, the neurons were bathed in TEA/Cs^+ saline which contained 1 μM TTX, and in which 6 mM Ca^{2+} was replaced by 6 mM Ba^{2+} (TTX/TEA/Cs^+/Ba^{2+}-saline). To record the inward current in *b* and Figs 2–4, the neurons were held at −50 mV and depolarized to 0 mV for 60 ms. CCK8 sulphate (1 mM stock solution in 1 M Tris-Cl, *p*H 7.8 was stored at −20 °C and a new sample used in each experiment.

The intracellular mechanisms which might mediate the effect of CCK8 were further investigated by making prolonged repetitive injections of either cAMP or cGMP, but neither affected the inward Ca^{2+} current in either D2 or F77 neurons. Intracellular injection of EGTA, a Ca^{2+} chelator (Fig. 2*a*), both before and during the application of CCK8 (5 nM) either totally prevented or markedly attenuated the inward current decrease induced by the peptide. If the injection of EGTA was stopped either before or during washout of the peptide, a delayed but full effect of CCK8 was observed (not shown). Moreover, injection of EGTA had no effect when CCK8 had previously decreased the inward current. The efficacy of the EGTA injections was tested in parallel experiments in which intracellular

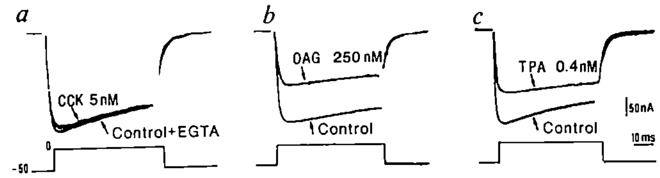

Fig. 2 The effect of CCK8 on the inward Ca^{2+} current of identified snail neurons is blocked by EGTA and is mimicked by activators of protein kinase C. *a*, A control inward current was recorded from a F77 neuron bathed in TTX/TEA/Cs^+/Ba^{2+}-saline. EGTA was pressure injected (pulses of 2 bars for 50 ms at 0.5 Hz) for 15 min. Five minutes after the onset of the EGTA injection, CCK8 (5 nM) was applied. The trace corresponds to two superimposed recordings obtained before and 7 min after CCK8 application. *b*, A control inward current was recorded from a F77 neuron bathed in TTX/TEA/Cs^+/Ba^{2+}-saline. Bath application of OAG (250 nM) markedly reduced the amplitude of the inward current. *c*, A control inward current was recorded from a different F77 neuron bathed in TTX/TEA/Cs^+/Ba^{2+}-saline. Bath application of phorbol ester TPA (0.4 nM) reduced the amplitude of the inward current. EGTA was pressure injected from a micropipette containing 0.25 M K_2-EGTA/60 mM KCl/1 mM HEPES (*p*H 7.4). OAG (10 mg ml^{-1}) and phorbol ester TPA (1 mg ml^{-1}) were dissolved in absolute ethanol and diluted in TTX/TEA/Cs^+/Ba^{2+}-saline. Scale bars 50 nA and 10 ms.

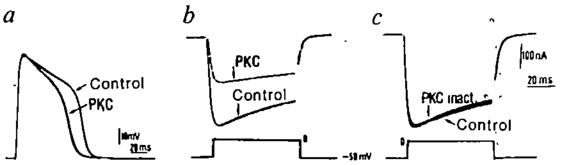

Fig. 3 Intracellular injection of protein kinase C mimics the effect of CCK8 on the Ca^{2+}-dependent action potential and on the inward Ca^{2+} current of identified snail neurons. *a*, A control Ca^{2+}-dependent action potential was recorded from a F77 neuron bathed in TEA/Cs^+ saline. Protein kinase C was then pressure injected (2 pulses of 2 bar for 10 ms). After 2-3 min, a decrease in the duration of the somatic spike (PKC) was observed. Scale bars 10 mV and 20 ms. *b*, A control inward current was recorded from a different F77 neuron bathed in TTX/TEA/Cs^+/Ba^{2+}-saline. Protein kinase C was then pressure injected (3 pulses of 2 bar for 10 ms). After 7 min, a marked decrease of the inward current (PKC) was observed. *c*, Pressure injection (50 pulses of 2 bar for 100 ms) of heat-inactivated protein kinase C in a different F77 neuron had no effect on the inward current. The trace corresponds to two superimposed recordings obtained before and 15 min after (PKC inact.) injection of the enzyme. Scale bars 100 nA and 20 ms. Protein kinase C was purified from bovine brain using a modification of the method of Kikkawa *et al.*[23]. The enzyme was totally inactivated by incubation at 60 °C for 12 min. Protein kinase C was pressure injected using siliconized micropipettes of 1-2 μm tip diameter.

injections of EGTA were shown to block the Ca^{2+}-dependent outward current evoked by repetitive depolarizing pulses in the identified neurons bathed in normal saline (not shown).

The EGTA experiments suggested a participation of cytosolic Ca^{2+} in mediating the effect of CCK8. Because inositol trisphosphate ($InsP_3$) has been shown to control the release of Ca^{2+} into the cytosol[11], we injected $InsP_3$ into the identified neurons. It had no consistent effects either on the basal inward current or on the CCK8-induced decrease of inward current. Cytosolic Ca^{2+} is also involved in the activation of protein kinase C[12], so possible involvement of this enzyme was examined. Bath application of oleoylacetylglycerol (OAG, 250 nM), a diacylglycerol analogue which activates protein kinase C[12], induced a marked and irreversible decrease in the amplitude of the inward current in F77 neurons (Fig. 2*b*). When the OAG concentration was increased to 60 μM its effect was maximal, the amplitude of the inward current being reduced by 80%. In these conditions, addition of CCK8 to the bath was totally ineffective. In other experiments, application of the phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA, 0.4 nM), another potent activator of protein kinase C[12], also induced a marked and irreversible decrease in the amplitude of the inward current (Fig. 2*c*).

The effect of intracellular pressure injection of protein kinase C was investigated. In all neurons tested ($n = 6$) pressure injection of protein kinase C evoked a marked shortening of the Ca^{2+}-dependent somatic action potential (Fig. 3*a*). Pressure injection of the enzyme also evoked a marked and irreversible decrease of the inward current in all neurons tested ($n = 10$) (Fig. 3*b*). In contrast, repeated pressure injection of heat-inactivated protein kinase C did not affect the amplitude of the inward current in the identified neurons ($n = 7$) (Fig. 3*c*).

Intracellular injection of protein kinase C also enhanced the effectiveness of low concentrations of CCK8. In control F77 neurons ($n = 5$), CCK8 (1 nM) evoked a 21.6±1.6% (mean± s.d.) decrease in the inward current of the identified neurons (Fig. 4*a*). Pressure injection of a fivefold diluted sample of the protein kinase C preparation had no effect by itself on the Ca^{2+} current (Fig. 4*b*). However, in neurons ($n = 5$) which had been injected with the diluted sample of protein kinase C, CCK8 (1 nM) induced a 35.6±3.4% (mean ±s.d.) decrease in the amplitude of the inward current (Fig. 4*b*), a decrease significantly greater ($P < 0.001$, Student's test) than that obtained by applying CCK8 alone.

We propose the following mechanism to account for the modulation by CCK8 of the inward Ca^{2+} current of D2 and F77 neurons: the peptide binds to receptors on the plasma membrane of the neurons, causing the formation of diacylglycerol from inositol phosopholipids; diacylglycerol, in the presence of an adequate cytosolic Ca^{2+} concentration, activates protein kinase C which in turn phosphorylates Ca^{2+}-channel(s) or some protein associated with them. This interpretation is supported by the following findings. (1) CCK8 acts on identified snail neurons at concentrations similar to those at which it binds to receptors in both pancreatic[13] and brain[14] membranes. (2) Two activators of protein kinase C (OAG and the phorbol ester TPA) mimic the effect of CCK8 on the Ca^{2+} current. (3) Intracellular injection of protein kinase C both mimics and enhances the CCK8-induced decrease of Ca^{2+} current. (4) Intracellular injections of EGTA prevent the CCK8-induced decrease of Ca^{2+} current. This effect of EGTA may be explained by the fact that protein kinase C, even in the presence of diacylglycerol, requires low concentrations of Ca^{2+} for activity[12].

The decrease in inward Ca^{2+} current induced by CCK8 appears to be irreversible or, at least, reversible only very slowly. In the same identified snail neurons, a brief application of dopamine evokes a decrease of the Ca^{2+} current which is rapidly reversible and not affected by the intracellular injection of EGTA[15]. These results suggest that two distinct mechanisms

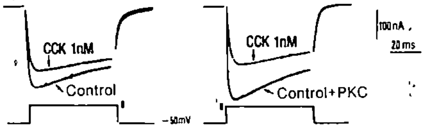

Fig. 4 Injection of protein kinase C enhances the effectiveness of CCK8 on the inward Ca^{2+} current of identified snail neurons. *a*, Inward currents recorded from a F77 neuron bathed in TTX/TEA/Cs^+/Ba^{2+}-saline before and 7 min after exposure to 1 nM CCK8; the peptide reduced the inward current by 20%. *b*, Protein kinase C, diluted fivefold, was pressure injected into a different F77 neuron (50 pulses of 2 bar for 20 ms) before bath application of CCK8. This concentration of protein kinase C had no effect on the inward current; the control trace actually corresponds to two superimposed recordings obtained before and 10 min after injection of protein kinase C. CCK8 (1 nM) was then applied; the inward current measured 7 min after the peptide application was decreased by 35%. Scale bars 100 nA, 20 ms.

exist for the regulation of the Ca^{2+} current, one possibly involved in long-term and the other in short-term changes of neuronal excitability.

Activators of protein kinase C have previously been found to increase the Ca^{2+} current in *Aplysia* bag cells[4,16], and to decrease reversibly the Ca^{2+} current in cultured chick embryo dorsal root ganglion neurons[5]. However, the neurotransmitters which activate protein kinase C are not known in any of these systems. The opposite actions of protein kinase C in these different systems suggest that distinct types of Ca^{2+}-channels may be phosphorylated by the enzyme. Protein kinase C has also been reported to decrease two different K^+-currents in photoreceptors of the mollusc *Hermissenda* [17] and a Cl^--current in mammalian hippocampal neurons[18]. More recently, it has been reported [19] that bradykinin depresses the M current, a particular K^+-current, in a neuroblastoma hybrid cell line in culture and that this effect is mimicked by activators of protein kinase C. CCK8 has been shown to depolarize and/or excite mammalian hippocampal pyramidal cells[20], dentate gyrus [21] and spinal dorsal neurons [22]. However, the mechanism of action of CCK8 in these neurons is not known. We have provided evidence in this study suggesting that, at least in identified neurons of *Helix aspersa*, CCK8 modulates the Ca^{2+} current by activating protein kinase C.

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Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers?

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Endothelial cells line the inner surface of blood vessels and act as the main barrier to the passage of cells and large molecules from the blood stream to the tissues. Recent interest in the part played by the endothelium in regulating vascular tone has focused on the synthesis and secretion of prostacyclin[1,2] and an endothelium-derived relaxing factor[3,4]. Endothelial cells respond to blood-borne agonists[5], but how the endothelium senses and responds to mechanical forces generated by the flow of blood under pressure is not known[6–12]. Here we report patch-clamp recordings of ion channel activity from cell-attached membrane patches on aortic endothelial cells. In most of the patches examined, we observed unitary inward currents associated with the opening of a cation-selective channel (~40 pS in standard saline). The channel is permeable to Ca^{2+} and its opening frequency increases when the membrane is stretched by applying suction through the patch electrode. The presence of mechanotransducing ion channels[13–15] in endothelial cells may help explain how the endothelium mediates vascular responses to haemodynamic stresses.

Figure 1 shows the pattern of single-channel activity recorded from a cell-attached patch, with physiological saline in both the patch electrode and in the bath. The records in Fig. 1*a* were obtained with the patch potential displaced −19 mV from the resting potential. Channel openings appeared as square pulses of inward current of about 1.4 pA. The relationship between the holding potential and the amplitude of the unitary current is shown in Fig. 1*b*. The $I-V$ relation was linear over the voltage range examined with a slope conductance of 39 ± 3.3 pS ($n = 7$).

An analysis of the kinetics of channel activity recorded at a constant voltage showed gating involved multiple open and closed states. At least two exponentials were needed to fit the open time histogram (Fig. 1*c*), corresponding to short and long openings in the single channel records (Fig. 1*a*). The distribution of closed times was not well resolved (Fig. 1*d*); there were at least two short-lived closed states corresponding to very brief gaps within long bursts and longer closings separating openings of both types. In addition, a much longer closed state having a time constant of seconds accounted for extended periods of silence between episodes of activity such as the one shown in Fig. 1*a*. There were too few of these very long closings to contribute a significant component to the histogram of closed times.

To show the changes in channel activity on a longer time scale, the number of openings in consecutive and non-overlapping time intervals was counted and plotted as a function of time as shown in Fig. 1*e*. Channel activity was high initially, but declined and remained low, except for the peak of activity in the interval marked with the bar (the records in Fig. 1*a* are from this period). The number of openings during this period was significantly greater than the expected mean calculated for the entire recording.

The rapid decline in channel activity after the electrode sealed to the membrane suggested channels might be responding to deformation of the membrane as it was sucked into the electrode as a seal was formed. Figure 2 shows the effect of suction applied by the electrode several minutes after seal formation. In this and subsequent experiments, the bathing solution contained isotonic potassium aspartate to set the cell resting potential to 0 mV (Fig. 2 legend). Three consecutive traces are shown with the patch potential held at −57 mV. When suction was applied through the electrode to the patch (indicated with arrows), openings of unitary amplitude occurred in rapid succession throughout the stimulus. We have not yet analysed the kinetics of the response to suction. Inspecting the current records suggests suction increased channel opening probability by increas-

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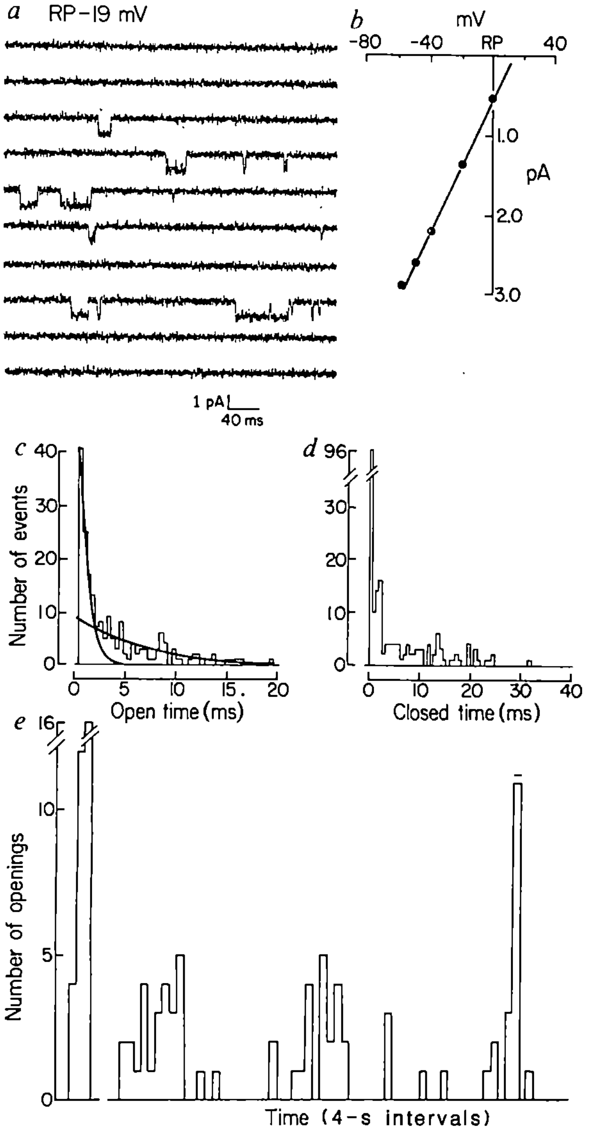

Fig. 1 Single-channel activity recorded from a cell-attached patch on an aortic endothelial cell. Both the bath and patch electrode contained standard saline (below). *a*, Spontaneous channel activity recorded from a cell-attached patch. Patch holding potential = resting potential −19 mV. *b*, Amplitude of the unitary event as a function of the applied potential. Although the resting membrane potential is unknown in this experiment, it was estimated to be about −30 mV by comparing the reversal potential with the reversal potential measured in other experiments where the cell was bathed in isotonic potassium aspartate and the membrane potential was known to be zero. *c*, *d*, Histograms of open and closed times, respectively. In the open time histogram in *c*, the fast exponential was drawn with a time constant of 0.7 ms, and the slower exponential with a time constant of 6.0 ms: both were fitted by eye. *e*, Channel activity during the first 5 min of the experiment. Zero time corresponds to the first records obtained after establishing a seal between the electrode and the membrane. The numbers of openings in consecutive and non-overlapping time intervals of 4.096 s were counted (discarding openings of two sample points or less) and plotted as a function of interval number. Records in *a* are from the interval marked with the horizontal bar. Cell 12-21-4.

Methods. Endothelial cells were isolated from neonatal pig aorta and cultured as described[22]. Cells were passaged twice weekly. Only cells from the first 3-4 passages were used for experiments. Standard saline contained 150 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES and was adjusted to *p*H 7.5 with NaOH. Current signals were recorded on magnetic tape. For analysis, currents were filtered with an eight-pole Bessel filter with a cut-off frequency (−3 dB) of 1 kHz and sampled at 5 kHz onto the hard disk of a PDP-11/23 laboratory computer. Experiments were done at room temperature (~21 °C).

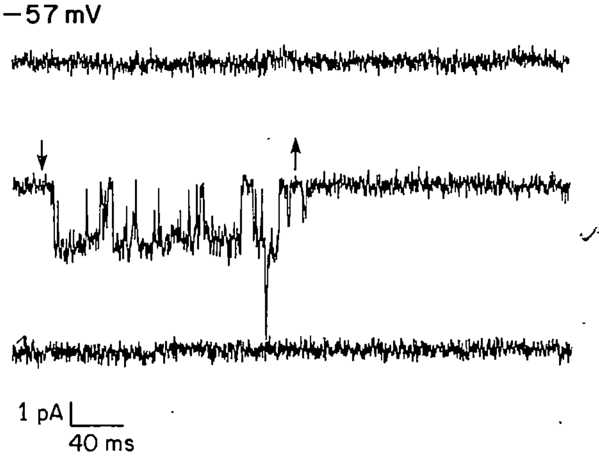

Fig. 2 Response of patch current to suction applied to the membrane through the electrode. In these experiments, suction pulses of 10-20 mm Hg (1.3-2.7 dyn cm^{-2}) were used to activate channel opening. Arrows, approximate onset and termination of the pulse of suction applied to the electrode through the suction line. The current records are from consecutive sweeps. The electrode contained standard saline. The bathing solution contained 150 mM KOH, 150 mM aspartic acid, 5 mM $MgCl_2$, 1 mM EGTA, 10 mM glucose, and 10 mM HEPES adjusted to *p*H 7.5 with KOH. In the isotonic K^+ bathing solution the resting membrane potential is close to 0 mV, because excising the patch from the cell did not measurably alter the amplitude of the unitary current[23]. Changing the bathing solution from physiological saline to isotonic potassium aspartate had no effect on channel activity or cell viability. Note the opening of a second channel of unitary amplitude. Holding potential was −57 mV. Cell 12-20-2.

ing the burst frequency, perhaps by shortening the duration of the longer closed periods between openings[14].

Applying suction was the only stimulus we found that opened channels. Changing the membrane potential over a wide range failed to open channels in quiescent cells. Excising the patch into a bathing solution containing either 2 mM Ca^{2+} or 5 mM EGTA (no added Ca^{2+}) or including 2 mM Ca^{2+} in the electrode solution did not alter channel activity. On the other hand, suction had no effect on the activity of a 60 pS K^+ channel we have seen in other patches (data not shown).

We examined the permeability of the channel to various monovalent cations. Channels were identified by their response to suction, which was similar to that shown in Fig. 2. With isotonic KCl in the electrode, the channel conductance was 56.0 ± 11.0 pS and current reversed at −5.0 ± 3.5 mV ($n = 5$). With standard Na^+-containing saline in the electrode, the reversal potential was +8.6 ± 16.0 mV ($n = 5$). One experiment with isotonic CsCl in the electrode gave a conductance of 27 pS and a reversal potential of +4.4 mV. Replacing half the chloride with asparate in the electrode solution or excising the patch so that its cytoplasmic surface was exposed to the isotonic potassium aspartate bath solution did not measurably alter either the conductance or the reversal potential, suggesting the channel is not permeable to chloride.

Figure 3 shows an experiment in which the patch electrode contained 110 mM $CaCl_2$. Channel activity in response to suction consisted of a rapid succession of brief openings and closings (arrows); here the inward current is presumably carried by Ca^{2+}. The relationship between the patch potential and amplitude of the unitary current is shown in Fig. 3*b*. The slope conductance was 19.1 ± 3.0 pS and the reversal potential was 17 ± 13.0 mV ($n = 5$). Calculation of the relative permeability to Ca^{2+} and Na^+ from the reversal potential measurements with the constant field equation[15-18] indicated the channel is somewhat more permeable to Ca^{2+} than to Na^+ ($P_{Ca^{2+}}/P_{Na^+} = 6.0$, range 1.2-8.4).

This paper reports the first measurements of ion-channel

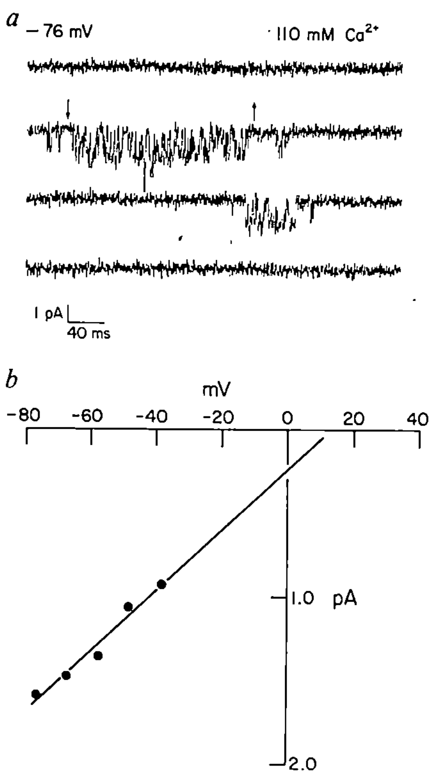

Fig. 3. Currents carried by Ca^{2+} through single stretch-activated channels. Records in *a* are from consecutive sweeps; arrows, approximate duration of the suction pulse. *b*, Amplitude of the single channel current plotted as a function of the applied voltage. Pipette solution contained 110 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES adjusted to *p*H 7.5 with tetraethylammonium hydroxide. Cell resting potential zeroed with isotonic potassium aspartate. Holding potential was −76 mV. Cell 12-20-7.

activity in endothelial cells. The stretch-activated channel described here is similar to the channel in skeletal muscle described by Brehm *et al.*[13] and Guharay and Sachs[14,15]. Our conductance measurements agree with those reported by Guharay and Sachs, who also found the conductance to be larger in external K^+ than in Na^+ solutions. We found that the reversal potential is somewhat less sensitive to the species of monovalent cation. Our results also differ from those of Guharay and Sachs in that we find the histogram of open times has an additional slow component.

The results show that Ca^{2+} fluxes through stretch-activated channels in isotonic $CaCl_2$ are large enough for channel openings to be detected as unitary events. Although Na^+ would carry significant current under physiological conditions, sufficient Ca^{2+} may enter the cell to serve a second messenger function. Ca^{2+} mediates the synthesis and release of prostacyclin and endothelium-derived relaxing factor stimulated by various agonists[3,4,19]. The fact that an intact endothelium is required for arterial contraction in response to stretching[20,21] and for the vasodilation which occurs in response to shear stresses produced by the flow of blood over the endothelial surface[8,12] makes it tempting to speculate that the effects of blood pressure and flow are mediated by mechanotransducers of the type described here.

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Relaxin affects the release of oxytocin and vasopressin from the neurohypophysis

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The hormone relaxin (for review see refs 1 and 2) has recently been shown to inhibit not only uterine muscle contraction[3], but also the release of oxytocin into the plasma. Intravenous injection of porcine relaxin in anaesthetized lactating rats inhibits milk ejection and injection of relaxin into the cerebral ventricles disturbs the pattern of the milk ejection reflex[4]. Recent experiments performed *in vivo* indicate that relaxin might act not only in the uterus, but also in the hypothalamus and possibly in the neurohypophysis[5]. We tested this hypothesis *in vitro* by studying the effect of relaxin on hormone release from isolated neural lobes of the pituitary and isolated neurosecretory nerve endings of the neurohypophysis from the rat. We report here that relaxin has a dual effect on neurohypophysial hormone secretion. Under basal conditions, vasopressin and oxytocin release was inhibited by relaxin but, when the nerve endings were depolarized, vasopressin and oxytocin secretion was potentiated. We also found that relaxin acts at a stage before the increase in cytoplasmic free Ca^{2+} that is necessary for inducing hormone release, possibly by gating the calcium channel.

Figure 1 illustrates the time course of vasopressin (AVP) and oxytocin (OT) release from isolated nerve terminals in the presence or absence of 10^{-7} M relaxin. An increase in hormone secretion was induced by depolarizing the isolated nerve terminals (neurosecretosomes) with 100 mM K^+ for 10 min. Relaxin decreased the basal AVP and OT release by 53% and 43% respectively (Table 1). On the other hand, relaxin greatly potentiated the K^+-evoked hormone release. The AVP release increased by 98% and that of OT by 59% (Table 1).

Figure 2 shows the dose-response curves of K^+-evoked (Fig. 2*a*) and basal (Fig. 2*b*) AVP and OT release obtained from experiments similar to those described in Fig. 1. For the evoked hormone release, half-maximal activation of AVP and OT release occurred at concentrations of 13 nM and 25 nM relaxin respectively. For release under resting conditions, the K_D was 1 nM for AVP and 2.5 nM for OT. These values might be overestimated, because relaxin binds to plastic material such as

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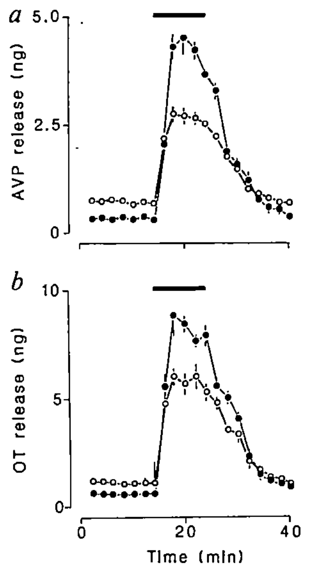

Fig. 1 Effects of relaxin on oxytocin and vasopressin release from isolated neurosecretory nerve terminals stimulated with elevated potassium concentration. The nerve endings were perfused with saline in the presence of 10^{-7} M relaxin. Stimulation of release was induced, during the time indicated by the heavy bar, with 100 mM potassium, choline being reduced accordingly. The amounts of vasopressin (*a*) and oxytocin (*b*) released in the perfusate were measured by radioimmunoassay. ●, Amounts released in the presence of relaxin; ○, controls. Note that under basal conditions relaxin decreases the amount of hormone released. The results are the mean ±s.e.m. of 4 experiments. Standard errors larger than the symbols used are shown as bars.

Methods. The isolated nerve endings were prepared by homogenizing two neural lobes isolated from male Wistar rats (body weight 250-300g) in 1 ml of 0.27 M sucrose containing 10 μM EGTA and buffered with 10 mM HEPES at *p*H 7.2 (ref. 9). The homogenate was centrifuged at 600*g* for 4 min and the resulting supernatant was centrifuged at 3,400*g* for 15 min. The pellet contained mostly isolated nerve endings and swellings as checked by electron microscopy. The pellet was resuspended in normal saline containing (mM): NaCl, 140; KCl, 5; $NaHCO_3$, 5; $CaCl_2$, 2.2; $MgCl_2$, 1; glucose, 10; HEPES, 10; *p*H 7.2 and to which BSA, at a final concentration of 0.01%, was added. The nerve endings were loaded onto a 0.22 μM Millipore filter and perfused with saline for 35 min at a flow rate of 50 μl min^{-1}. The neurosecretosomes were then perfused with Na-free saline containing an equiosmolar quantity of choline and the flow rate was increased to 100 μl min^{-1}. Collection of the perfusate started 45 min after loading.

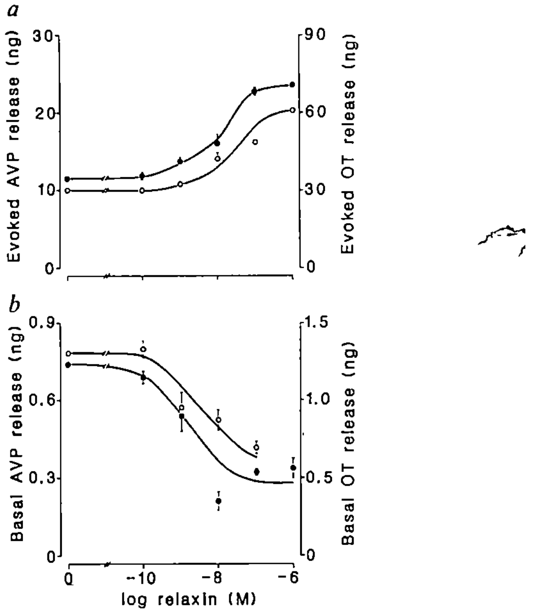

Fig. 2 The effects of increasing concentrations of relaxin on evoked and basal oxytocin and vasopressin release from isolated nerve terminals. The neurosecretosomes were perfused with Na-free saline and depolarized with 100 mM K^+ as in Fig. 1. The evoked release (*a*) of (●) AVP and (○) oxytocin was calculated by subtracting the mean basal release (*b*), determined in the fractions preceding the onset of the stimulus, from the amount of hormone found in each fraction. Relaxin, at the different concentrations indicated on the abcissa, was present before and during the K^+ stimulation. Values are mean ±s.e.m. ($n = 4$).

that used in the perfusion system (G. Bryant-Greenwood and C. Bagnell, personal communication). Thus, *in vivo*, relaxin might modulate the release of neurohypophysial hormones at even lower concentrations.

In another series of experiments, isolated neural lobes, separated from the pars intermedia, were electrically stimulated with a pattern of firing mimicking the electrical activity of the magnocellular neurons of the hypothalamus[6]. Relaxin (10^{-7} M) potentiated both AVP and OT release when hormone secretion was triggered either with AVP-like bursts (Fig. 3; Table 1) or with electrical discharges mimicking the activity of oxytocin-containing cells during lactation (Table 1). In the presence of relaxin, the firing pattern of vasopressin-containing cells potentiated the release of AVP by 119% and that of OT by 79% (Table 1). Potentiation of release by relaxin also occurred following stimulation with OT-like discharges. At a relaxin concentration of 10^{-7} M, AVP and OT secretion was enhanced by 88% and 69% respectively (Table 1). Finally, we tested the effect of relaxin on hormone release from isolated neural lobes stimulated with a pattern mimicking the electrical activity of a cell with a slow firing rate. This type of firing can be observed in cells under resting conditions[7]. The mean firing rate was only 3.9 Hz, but the discharges were made up of small bursts containing four spikes separated by intervals of 15 ms. Thus, although the mean firing rate was 3.9 Hz the frequency of firing within the burst was much higher. The neural lobes were stimulated for 20 min and this gave rise to an increased hormone release which was, for AVP and OT, potentiated in the presence of relaxin (10^{-7} M) by 132% and 69% respectively (Table 1). Furthermore, as shown

Table 1 The effects of porcine relaxin on release of vasopressin and oxytocin from isolated nerve terminals and neural lobes

	Vasopressin		Oxytocin	
	Control	+Relaxin (10^{-7} M)	Control	+Relaxin (10^{-7} M)
Nerve endings:				
Basal	0.37±0.02 (4)	0.17±0.01 (4)	0.61±0.07 (4)	0.35±0.04 (4)
K^+-induced secretion	11.50±0.50 (4)	22.70±0.60 (4)	29.90±1.00 (4)	47.40±1.30 (4)
Neural lobe:				
Basal	0.11±0.01 (18)	0.07±0.003 (15)	0.30±0.03 (18)	0.08±0.005 (15)
'AVP-like' bursts	2.75±0.14 (5)	5.54±0.14 (5)	5.00±0.18 (5)	8.94±0.18 (5)
'OT-like' discharges	0.56±0.06 (5)	1.05±0.07 (5)	2.47±0.17 (5)	4.31±0.08 (4)
'Slow-firing' cell	1.07±0.06 (8)	2.48±0.10 (8)	1.78±0.09 (8)	3.00±0.12 (5)

Basal secretion and evoked hormone release are expressed as ng min^{-1} and ng respectively. The results are given as mean ±s.e.m. with the number of experiments indicated in parentheses.

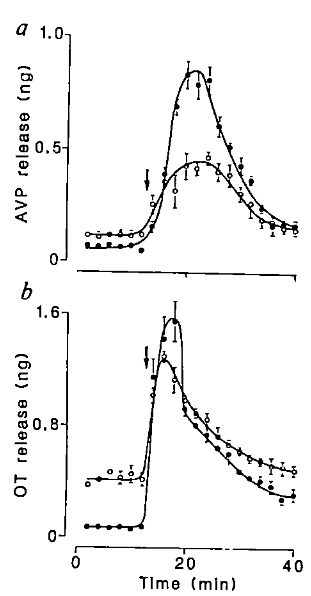

Fig. 3 Effect of relaxin on oxytocin and vasopressin release from electrically stimulated rat neural lobe. The neural lobes were stimulated with burst patterns mimicking the electrical activity of vasopressin-containing cells during haemorrhage. Arrows, onset of stimulation. AVP, (*a*); and OT, (*b*), in the perfusate were measured by radioimmunoassays. Values are the mean ±s.e.m. ($n=5$).

Methods. The neural lobes were obtained from male rats (body weight 250–300g) and the pars intermedia was carefully dissected out[9]. The neural lobe was impaled on one of a pair of electrodes which were inserted in a small perspex chamber which had an internal volume of about 50 µl. The gland was continuously perfused with normal saline (see Fig. 1 legend) in the presence or absence of relaxin (10^{-7} M). Fractions of 2 min duration (100 µl min^{-1}) were collected 45 min after the onset of the perfusion. The neurohypophyses were stimulated electrically with biphasic electrical pulses which had a pattern of discharge similar to the bursting activity of AVP cells[6]. Briefly, the electrical activity of a vasopressin cell recorded *in vivo* was used as a command pulse to trigger the stimulator and thus generate a series of pulses (2 ms duration, 4 mA, biphasic) similar to the bursting pattern observed *in vivo*. The neural lobes were stimulated with four 'AVP-like' bursts separated by intervals of 21 s.

above on the isolated nerve endings, relaxin considerably decreased AVP and OT secretion from the neurohypophysis under resting conditions (Table 1).

Naloxone, the function of which in the neurohypophysial release mechanism is still very controversial[8,9], did not modify the potentiating effect of relaxin. In the presence of 10^{-7} M relaxin, the 100 mM K^+-evoked hormone release from the neurosecretosomes was, in the presence and absence of 10^{-5} M naloxone, 24.3±0.3 and 22.7±0.6 ng for AVP, and 44.1±2.1 and 47.4±1.3 ng for OT. Furthermore, naloxone did not prevent the relaxin-induced inhibition of AVP and OT release (data not shown). This shows that relaxin does not interact, at least in the neurohypophysis, with a naloxone-sensitive opiate receptor.

To see if relaxin acts after the increase in ionized cytoplasmic concentration induced by the activation of voltage-dependent calcium channels, it was added before and during a period when internal calcium was artificially increased without depolarising the membrane. This involves making the neurosecretosomes permeable with digitonin in a medium containing micromolar concentrations of calcium[10]. Briefly, the nerve endings were perfused with normal saline, then with Ca-free medium of the following composition (mM): potassium glutamate, 130; $MgCl_2$, 2; EGTA, 2; glucose, 10; PIPES, 20. The medium contained 0.01% bovine serum albumin (BSA) and its *p*H was adjusted to 6.8. The neurosecretosomes were permeabilized, for a period of 10 min, with 1 µM digitonin in the presence of 1.1 µM Ca and 1 mM ATP. The Ca-dependent hormone release was calculated by subtracting the amount released in the presence of calcium from that secreted after permeabilization in a Ca-free medium. The Ca-independent release corresponded to $<10\%$ of the total amount secreted, suggesting that, after permeabilization, only a small amount of hormone was released by a non-specific mechanism. Relaxin did not modify the total evoked release of AVP and OT. In controls, the AVP and OT levels were 3.20±0.07 ng ($n=4$) and 6.20±0.77 ng ($n=4$) respectively. In the presence of relaxin (10^{-7} M) these values were 3.06±0.07 ng ($n=4$) and 5.84±0.27 ng ($n=4$) respectively. Thus relaxin does not act at a stage which couples the increased ionized cytoplasmic calcium concentration to the mechanism of exocytosis *per se* but interferes with the Ca entry preceding the release of hormone. Figure 4 shows the effects of different concentrations of relaxin on AVP release from neurosecretosomes in the presence of the calcium channel blocking agent nitrendipine. This dihydropyridine molecule is a potent antagonist of the depolarization-induced hormone release from isolated neurohypophysial nerve endings[11]. From its action on calcium current in other preparations[12–14], it can reasonably be assumed that it blocks voltage-gated calcium channels in the neurosecretory nerve endings. Relaxin considerably inhibits the effect of nitrendipine on AVP and OT release. Experiments performed with different relaxin and nitrendipine concentrations (Fig. 4) suggest a non-competitive inhibition by relaxin of the effect of nitrendipine on AVP release. Figure 4 also shows that nitrendipine did not affect the relaxin concentration necessary to induce half-maximal activation of hormone release but decreased, in a dose-dependent manner, the amount of AVP secreted after K^+-induction. We do not yet know if relaxin acts directly on the calcium channel at a binding site distinct from that of nitrendipine, or at a site associated but distinct from the calcium channel. Patch-clamp studies on neurosecretosomes[15,16] should help to answer this question soon. The dual effect of relaxin on basal and evoked hormone release makes it an attractive candidate for modulating the gating mechanism of the calcium channels in the plasma membrane of neurosecretory nerve terminals.

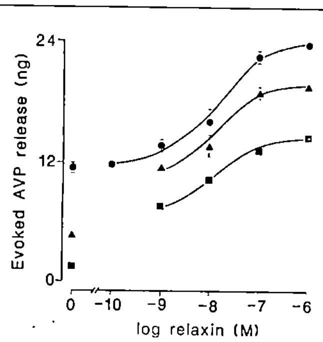

Fig. 4 Effects of relaxin on the depolarization-induced AVP release from nerve endings in the presence of nitrendipine. The neurosecretosomes were prepared as in Fig. 1 and perfused with different concentrations of relaxin. Hormone release was triggered with 100 mM K^+. Relaxin (10^{-7} M) and nitrendipine (4×10^{-6} M, ▲ or 15×10^{-6} M, ■) were present throughout the experiments. ●, Evoked AVP release in the absence of nitrendipine. Values are mean ±s.e.m. ($n=4$).

In conclusion, the present work shows that relaxin, a hormone produced *in vivo* by the ovary, can directly modulate the secretion of neuropeptides from the neural lobe of the pituitary. To our knowledge, this is the first direct evidence that a circulating hormone controls neuropeptide secretion from neurohypophysial nerve terminals. It is worth noting that relaxin decreases the milk ejection reflex when injected into anaesthetized lactating rats, presumably by acting within the central nervous system[4]. Further electrophysiological studies are needed to determine the effect of relaxin on the electrical activity of the magnocellular neurons. We do not yet know if the relaxin implicated in the release of neurohypophysial hormones is synthesized at the periphery or in the hypothalamus. The results demonstrate that relaxin acts at a step in the cascade leading to exocytosis which precedes the increase in free cytoplasmic Ca^{2+} concentration induced by depolarization of the plasma membrane. We suggest that in neurohypophysial nerve endings relaxin directly modulates the entry of calcium through Ca channels.

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Redesigning the body plan of *Drosophila* by ectopic expression of the homoeotic gene *Antennapedia*

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Genetic and molecular studies on the expression of *Antennapedia* (*Antp*) have suggested that this gene specifies mainly the second thoracic segment. On the basis of our molecular analysis of dominant gain-of-function mutants we have postulated that the transformation of antennae into second legs is due to the ectopic overexpression of the *Antp*$^+$ protein. This hypothesis was tested by inserting the complementary DNA encoding the normal *Antp* protein into a heat-shock expression vector and subsequent germline transformation. As predicted, heat induction at defined larval stages leads to the transformation of antennae into second legs. The dorsal part of the head can also be transformed into second thoracic structures (scutum) indicating that *Antp* indeed specifies the second thoracic segment. By ectopic overexpression of the *Antp* protein the body plan of the fruit fly can be altered in a predictable way.

Mutations at the homoeotic *Antennapedia* (*Antp*) locus affect the development of all three thoracic segments of *Drosophila*[1-4]. Recessive loss-of-function mutants, which correspond to the deletion of the locus, are late embryonic or larval lethals in which mainly the mesothoracic segment (T2) is transformed towards a prothoracic segment (T1) and head segments. The metathoracic segment (T3) is also partially transformed towards the meso- or prothoracic segment, that is in an anterior direction. Localization of *Antp*$^+$ transcripts by *in situ* hybridization[5,6] and of the *Antp*$^+$ protein by immunofluorescence[7] is consistent with the idea that *Antp*$^+$ mainly specifies the mesothoracic segment (T2), and also parts of T1 and T3, as both the RNA and the protein accumulate in these segments of the wild-type embryo. There is an even more striking class of mutants which are dominant and show a transformation of the adult antennae into second legs, towards the mesothorax, that is in the opposite direction to recessive loss-of-function mutants. These mutations are thought to represent a dominant gain-of-function, because they are dominant in triploids and their dominant phenotype can be 'reverted' by deleting the *Antp* gene[8,9]. An exceptional dominant gain-of-function mutant *Cephalothorax* (*Ctx*)[10] transforms the posterior dorsal part of the head into dorsal mesothoracic structures. However, this mutation results from a very complex chromosomal rearrangement with one breakpoint in the *Antp* locus[11] and only a single allele is known. Most of the mutations that lead to a transformation of antennae into second legs are chromosomal inversions[11,12]. The structural analysis of the *Antp* gene and the determination of the nucleotide sequence of the relevant parts[13] show that in all of the dominant gain-of-function mutants which have been analysed, the protein-coding region of *Antp* is not altered, but separated from its control region. Therefore, we have proposed that the dominant phenotype is not due to an altered gene product but is due rather to altered expression of the normal *Antp* protein. At least for the inversion *In*(3*R*)*Antp*^{73b}, we have been able to show that the chromosomal inversion results in the fusion of a 5′ control region and RNA coding sequences of a foreign gene to the *Antp*$^+$ protein-coding region giving rise to fusion transcripts[14]. As at least eight independent inversions are known[11-13] to produce antenna-to-leg transformations it seemed *a priori* unlikely that all these inversion events are due to fusion of the *Antp* protein-coding region to promoters which are expressed specifically in the antennal imaginal disk. This led to the speculation that the dominant gain-of-function phenotype may be caused by the ectopic overexpression of the *Antp* protein under the control of any promoter that would be expressed in the antennal imaginal disk at the appropriate time in development. Such a promoter might also be active in other tissues, but the effect would be limited to the antennae if the protein were expressed at the critical time when antennae are determined.

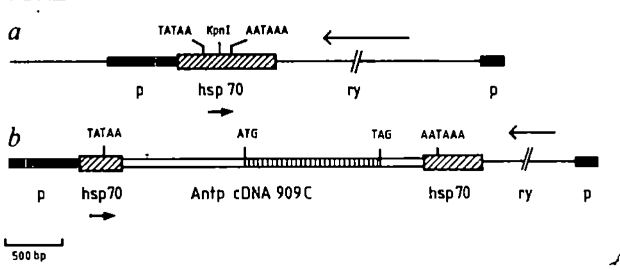

Fig. 1 Structure of the heat shock *Antp* fusion plasmids. *a*, Vector plasmid pHT 4. This vector was constructed by inserting the *hsp*70 promoter and the termination-processing sequences into Carnegie 20[24], a vector which contains the *rosy* (*ry*$^+$) gene flanked by the ends of the P-transposon[25]. The unique *Kpn*I site separates the *hsp* 70 leader from the *hsp* 70 trailer sequences. This site is suitable for cloning of passenger sequences, resulting in a fusion to the *hsp* 70 leader and expression under the heat-shock promoter. The leader fragment contains the first 95 base pairs (bp) of the *hsp* 70 leader and 250 bp upstream sequences. The trailer fragment contains the whole trailer sequence of *hsp* 70 and 350 bp of non transcribed 3′ flanking sequences. *b*, Heat shock *Antp* fusion plasmid pHTA. The 2.8-kb *Eco*RI fragment from *Antp* cDNA 909c[12,13] containing the whole open reading frame was made blunt ended with the Klenow fragment of *E. coli* DNA polymerase I and cloned into vector pHT4, which was linearized with *Kpn*I and made blunt ended by a T4 polymerase reaction.

To test this hypothesis of ectopic overexpression we constructed an artificial gene fusion in which the *Antp* protein-coding region is fused to the heat-inducible promoter of the heat-shock

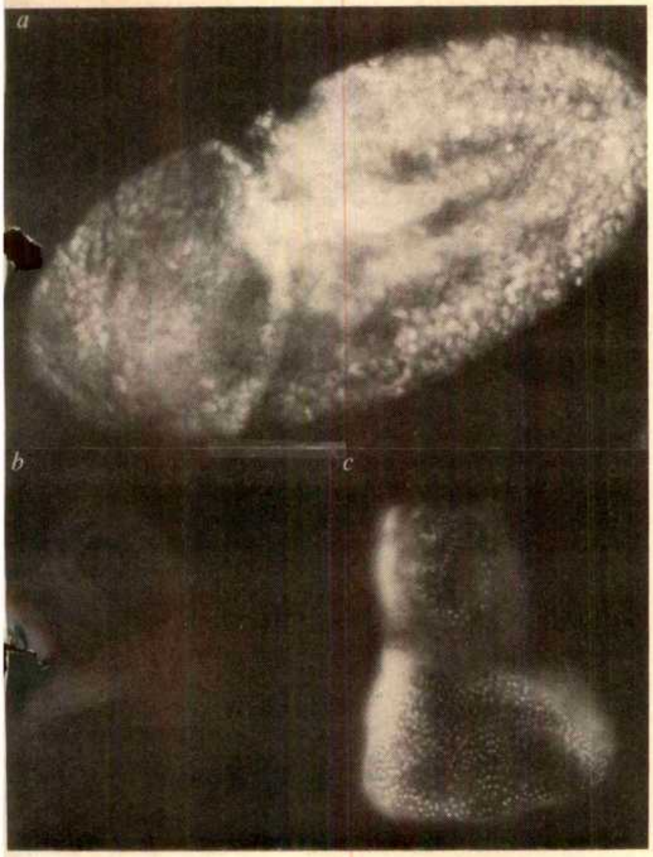

Fig. 2 *Antp* protein expression after heat induction. *a*, Embryos of transformant H4 were incubated for 7 h at 25 °C, heat shocked for 4 h at 37 °C, fixed, stained with anti-*Antp* aniserum[7] and mounted in toto. Note that the entire embryo is labelled and that the antigen is located in the nuclei. *b*,*c*, Eye-antennal disks of late third-instar larvae from the transformant H4 were fixed and stained as above, with and without heat induction. In the non-heat-shock control disk (*b*) only cytoplasmic background staining is detected, whereas all nuclei are labelled after a 2 h heat shock at 37 °C followed by a 2 h recovery period at 25 °C.

gene *hsp*70[15-17]. This fusion gene has been introduced into the germline of wild-type recipient flies by P-element mediated germline transformation. As the *Antp* gene is very large and comprises more than 100 kilobases (kb), an *Antp* cDNA was inserted between the heat-shock promoter *hsp*70 and its termination-processing sequences. For this purpose the *Antp* cDNA (909c) which contains the entire large open reading frame[13] was inserted into the *Kpn*1 site of the newly constructed pHT4 vector (Fig. 1*a*). The final construct, plasmid pHTA, used for germline transformation is shown in Fig. 1*b*. DNA of pHTA was coinjected with pπ25.7 w.c. helper plasmid into ry^{506} (ORM) recipient eggs[18]. Several independent transformed ry^+ fly lines were established. They have insertions on different chromosomes (lines H4, H26, H49 on the 3rd, H22, H36 on the 2nd and H21, H45 on the X-chromosome) and all of them are homozygous viable except for line H21, in which the insertion seems to have induced a lethal mutation. Line H4 was used for most of the experiments described below. Other lines showed similar results both in terms of protein accumulation and developmental response.

To determine whether the integrated fusion gene produced *Antp* protein under the control of the heat-shock promoter, the localization of the protein was monitored by immunofluorescence labelling with a polyclonal mouse anti-*Antp* serum. This antiserum, which was obtained by immunizing mice with a protein synthesized in *Escherichia coli* that contains parts of the *Antp* open reading frame, recognizes a nuclear antigen in late embryos which is mainly expressed in the three thoracic segments of the epidermis and the nervous system (T2, parts of T1 and T3) and corresponds to the *Antp*$^+$ protein[7]. Following exposure of transformant embryos to heat shock, *Antp*$^+$ protein can be detected in all nuclei of the embryo (Fig. 2). The nuclear localization was confirmed by double labelling with a nuclear dye (Hoechst 33258). These results demonstrate that these transformants produce an *Antp* protein which, after heat shock, is expressed throughout the embryo. The same result was obtained with larvae. Closer examination of the eye-antennal disks of transformed larvae (Fig. 2*b* and *c*) indicates that in the heat-shocked disks all nuclei are strongly labelled by the antiserum, whereas in the non-heat-shocked controls only weak non-specific staining of the cytoplasm can be detected.

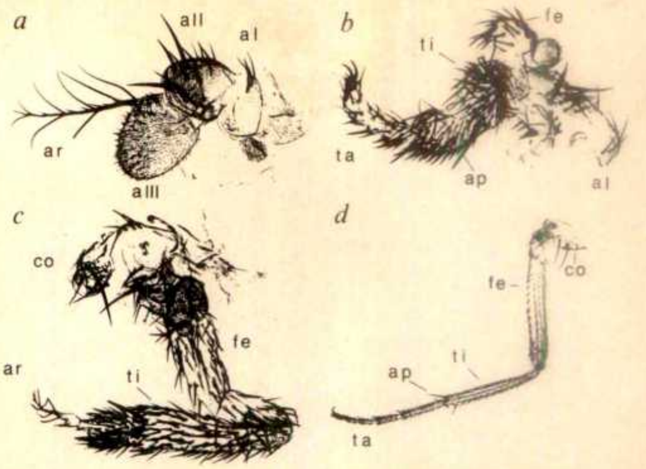

Fig. 3 Induction of *Antp* phenotype by heat shock. *a*, Normal antenna (×68). *b*, *c*, Two examples of antennae from heat-shocked flies. Heat shocks were for 2 h at 37 °C on the 4th and 5th days of development (3rd-instar larvae). The main parts of the antennae are transformed into leg structures. In *b*, the arista is completely transformed into tarsal structures including claws, whereas in *c* more proximal structures are transformed. The presence of the apical bristle indicates the mesothoracic identity of the leg (×22) (×68). *d*, Normal mesothoracic leg (×22). Abbreviations: aI, antennal segment I; aII, antennal segment II; aIII, antennal segment III; ar, arista; co, coxa; fe, femur; ti, tibia; ap, apical bristle; ta, tarsus.

The developmental effects of heat induction were tested by temperature shift experiments. At the normal temperature (25 °C) the transformed flies showed no obvious phenotypic alterations although the *hsp*70 promoter is known to be weakly active even at this temperature[19]. The effect of heat induction of *Antp* was examined by applying a heat shock at defined developmental stages. Eggs were collected in small tubes at 25 °C and allowed to develop at this temperature. A 1–2 h heat shock at 37 °C was applied at various stages and the tubes were returned to 25 °C to allow continued development. Figure 3 shows the results of experiments in which the transformed flies were exposed to heat shock during the third larval stage as described in the legend. In more than 50% of the heat-shocked flies, the antennae were clearly transformed into leg structures. On the basis of the bristle pattern, these leg structures correspond to those of the mesothoracic leg as indicated for example by the characteristic apical bristle on the tibia. In most cases the transformation is incomplete, but it corresponds exactly to those found in strong *Antp* mutant alleles. Therefore, heat-induced ectopic expression of the *Antp*$^+$ protein results in the formation of mesothoracic legs in place of antennae, that is in an alteration of the body plan as predicted by our hypothesis. No such transformations have been observed in control flies which were transformed with the vector pHT 4 alone without the *Antp* insert and heat shocked at the same stages of development.

Closer examination of the adult phenotype following heat shock revealed additional transformations. As shown in Fig. 4*a*

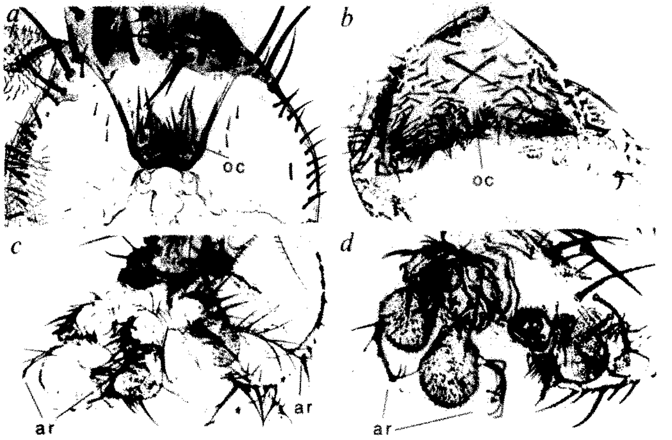

Fig. 4 Induction of *Cephalothorax* and *Dfd*-like phenotypes. *a*, Normal hind head with occiput (×52). *b*, Hind head from a fly, which was treated in the same way as in Fig. 3. Note the large symmetrical outgrowth of thoracic structures which resembles the *Cephalothorax* phenotype (×52). *c*, *d*, *Dfd*-like phenotype after heat shock during 1st and 2nd instar larval stage. In *c*, a complete additional antenna replaces one eye whereas in *d* parts of an antenna are duplicated as indicated by the presence of two aristae (×45). Abbreviations: ar, arista; oc, occiput.

and *b*, a region of the hindhead of heat-shocked flies is transformed into dorsal mesothoracic structures of the scutum. This phenotype corresponds to a strong *Ctx* phenotype and presumably represents another gain-of-function phenotype. It occurs at about the same frequency as the antenna-to-leg transformations. Other head defects and exceptional cases of antennal duplication have also been noted (Fig. 4*c* and *d*). These phenotypic effects are observed rarely. They resemble flies homozygous for the recessive *Deformed* allele (Dfd^{r-L}).[20]

The precise developmental time during which the antennae-to-leg transformation can be induced is critical. Heat shock during the first and second larval instar produces no apparent effects and if the heat shock is applied late in development, just before pupation, only a few leg bristles with their characteristic bract are induced. A heat shock during precisely defined embryonic stages results in complete lethality of the transformants. Such embryos fail to involute the head segments properly and also exhibit segmental transformations towards T2 that will be described elsewhere (G. Gibson, S.S. & W.J.G., in preparation).

These transformation experiments demonstrate that the earlier gene assignment based on various *Antp* mutants[11,12] was correct and that the 909c open reading frame encodes a functional *Antp* protein. They also show conclusively that *Antp* controls the developmental pathway leading to the second thoracic segment and specifies not only the ventral parts (the mesothoracic legs) but also dorsal parts (the scutum). We interpret the observation that ectopic expression of *Antp* protein can lead to a homoeotic segmental transformation in terms of a model which assumes the competitive regulatory interaction among the various homoeotic genes[21]. As shown by earlier experiments on transdetermination[22,23], the antennal disk seems to be a relatively weak spot in the genetic circuitry as a homoeotic transformation from antennae to leg can occur spontaneously during *in vivo* culture. After deciphering the genetic circuits that control development further, it should be possible to understand the principles underlying the specification of the body plan both in molecular and evolutionary terms.

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Homology between *Streptomyces* genes coding for synthesis of different polyketides used to clone antibiotic biosynthetic genes

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Many important antibiotics such as tetracyclines, erythromycin, adriamycin, monensin, rifamycin and avermectins are polyketides. In their biosynthesis, multifunctional synthases[1] catalyse iterated condensation of thio-esters derived from acetate, propionate or butyrate to yield aliphatic chains of varying length and carrying different alkyl substituents. Subsequent modifications, including aromatic or macrolide ring closure or specific methylations or glycosylations, generate further chemical diversity. It has been suggested that, if different polyketide synthases had a common evolutionary origin, cloned DNA coding for one synthase might be used as a hybridization probe for the isolation of others[2]. We show here that this is indeed possible. Study of a range of such synthase genes and their products should help to elucidate what determines the choice and order of condensation of different residues in polyketide assembly, and might yield, by *in vitro* recombination or mutagenesis, synthase genes capable of producing novel antibiotics[3]. Moreover, because genes for entire antibiotic pathways are usually clustered in *Streptomyces*[4], cloned polyketide synthase genes are valuable in giving access to groups of linked biosynthetic genes.

The complete cluster of genes for actinorhodin biosynthesis was recently cloned from *Streptomyces coelicolor*[2]. By complementation tests[5] eight genes, identified using blocked mutants classified on co-synthetic and chemical criteria[6,7], were located on the cloned DNA. Class I and III mutants are blocked at the earliest steps in actinorhodin biosynthesis, because they convert intermediates secreted by mutants of all other co-synthesizing

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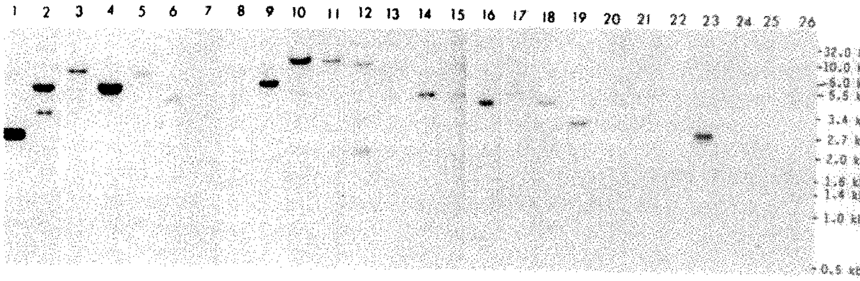

Fig. 1 Southern hybridization of an *act*I probe to restriction digests (*Bgl*II for samples 7, 13, 14 and 24 and *Bam*HI for the other samples) of genomic DNA from various antibiotic-producing streptomycetes. Lane, organisms and antibiotic as follows: 1, *S. coelicolor* A3(2) (actinorhodin); 2, *S. violaceoruber* Tü22 (granaticin); 3, *S.* sp. AM-7161 (medermycin); 4, *S. tanashiensis* NRRL 3215 (kalafungin); 5, *S. glaucescens* ETH 22794 (tetracenomycin); 6, *S. peucetius* var. *caesius* IMRU3920 (adriamycin); 7, *S. rimosus* (Pfizer) (oxytetracycline); 8, *S. erythreus* NRRL2338 (erythromycin); 9, *S. ambofaciens* ATCC15154 (spiramycin); 10, *S. fradiae* (Lilly) (tylosin); 11, *S. venezuelae* ATCC15068 (pikromycin, methymycin); 12, *S. antibioticus* ATCC 11891 (oleandomycin); 13, *S. griseus* IMRU3570 (candicidin); 14, *S. griseus* ETH A7796 (nonactin); 15, *S. albus* ATCC21838 (salinomycin); 16, *S. hygroscopicus* spp. *aureolacrimosus* (milbemycin); 17, *S. lasaliensis* NRRL3382R (lasalocid); 18, *S. cinnamonensis* (Lilly) (monensin); 19, *S. parvulus* ATCC12434 (actinomycin); 20, *S. curacoi* DSM40107 (curamycin); 21, *S. antiboticus* IMRU3720 (actinomycin); 22, *S. fradiae* NRRL B3357 (phosphomycin); 23, *S. venezuelae* UC2374 (chloramphenicol); 24, *S. griseus* ATCC10137 (streptomycin); 25, *S. albus* P; 26, *S. albus* G (salgiomycin). Washing was in 4 changes of 0.2×SSC, 0.1% SDS, 70 °C. (The second, weak, band of ~6 kb in the 'control' hybridization with *S. coelicolor* DNA in lane 1 represents a fragment of the DNA found to give rise to a brown pigment when introduced into *S. lividans*[12] and is distinct from the *act* DNA.) The *act*I probe was pIJ2345, which is pBR329[13] carrying the *Bam*HI fragment between sites 14–17 (Ref. 5). (In similar experiments with *act*III the probe was pIJ2346, which is pBR329 carrying the *Bam*HI fragment between sites 13–14.)

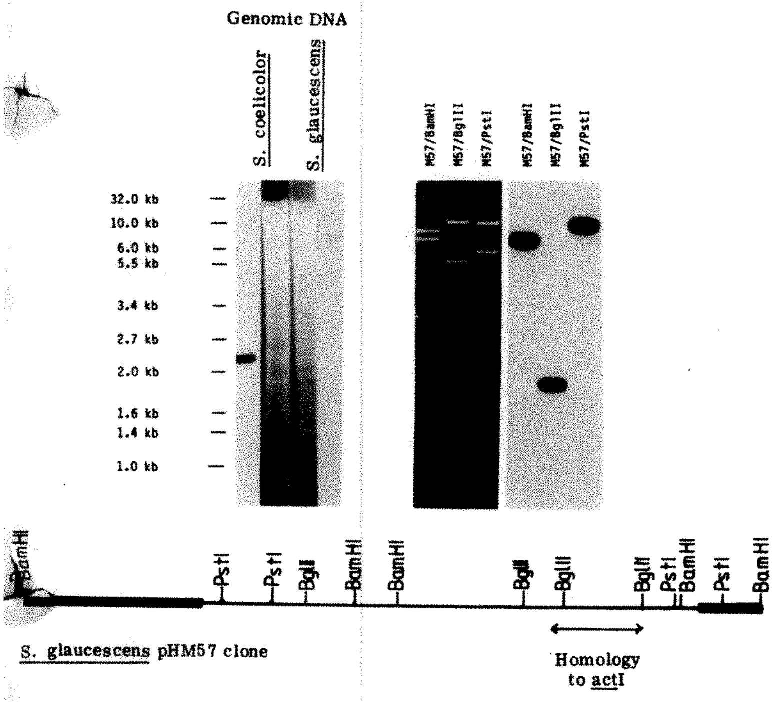

Fig. 2 Above, Southern hybridization of an *act*I probe (see Fig. 1 legend) with (left) *Bam*HI-digested genomic DNA of *S. coelicolor* and *S. glaucescens* and (right) digests of pHM57[8] carrying *S. glaucescens* tetracenomycin biosynthetic genes cloned in pIJ702[14]. Below, restriction map of pHM57 showing the limits of homology with the *act*I probe deduced from the experiment. Heavy lines represent pIJ702. (Note that pHM57 carries the early genes for tetracenomycin biosynthesis; pHM152, which partially overlaps pHM57 and carries the late genes[8], showed no hybridization with the *act* DNA.)

classes to actinorhodin but secrete no material capable of conversion by other mutants. Class VII mutants, blocked at the next identified step of the pathway, accumulate a shunt product derived from a complete polyketide chain[7]; thus gene VII acts after polyketide chain assembly. The amino-acid sequence of the *act*III gene product (derived from the DNA sequence) is similar to those of ribitol dehydrogenase from *Klebsiella aerogenes* and some other dehydrogenases/reductases (S.E.H., unpublished data). This is significant in the light of the finding that 6-methylsalicylic acid synthase from *Penicillium patulum* is a complex that includes a NADPH-linked reductase acting at a specific step in polyketide assembly[1]. Thus the *act*I and *act*III gene products appear to be components of the actinorhodin polyketide synthase: *act*III would code for a reductase and *act*I probably for the condensing enzyme itself.

DNA probes for *act*I and *act*III were hybridized at high stringency to restriction digests of genomic DNA from *S. coelicolor* (as control) and 25 other streptomycetes. With the *act*I probe, hybridizing bands of varying intensity were seen (Fig. 1) in the DNA from 14 of 18 known polyketide-producers, including those making anthracyclines (tetracenomycin, adriamycin), macrolides (spiramycin, tylosin, pikromycin, oleandomycin), polyethers (nonactin, salinomycin, lasalocid, monensin), and milbemycin as well as the isochromanequinone class (granaticin, medermycin, kalafungin) to which actinorhodin belongs (particularly strong hybridization was seen with two of these); only strains producing oxytetracycline, erythromycin, candicidin and curamycin showed no bands. Of the seven strains not known to produce polyketides, a chloramphenicol-producing *S. venezuelae* and actinomycin-producing *S. parvulus* and *S. antibioticus* strains showed hybridization (Fig. 1). With the *act*III probe, hybridizing bands were seen with the same set of strains as with the *act*I probe, except that in the tetracenomycin-producing *S. glaucescens* no band hybridizing with *act*III was revealed.

These results demonstrate a correlation, albeit incomplete, between polyketide production and the presence of DNA sequences partially homologous with the actinorhodin polyketide synthase probes. To see if some of the homologous sequences indeed represented polyketide synthase genes, previously cloned DNA for tetracenomycin C and oxytetracycline biosynthesis[8,9] was probed with *act*I and *act*III. Southern hybridization between the *act*I probe and clones carrying the entire set of tetracenomycin C genes (*tcm*)[8] revealed a segment of ~2 kilobases (kb) of homologous *tcm* DNA (Fig. 2), accounting for the hybridization to *act*I in genomic digests of *S. glaucescens*

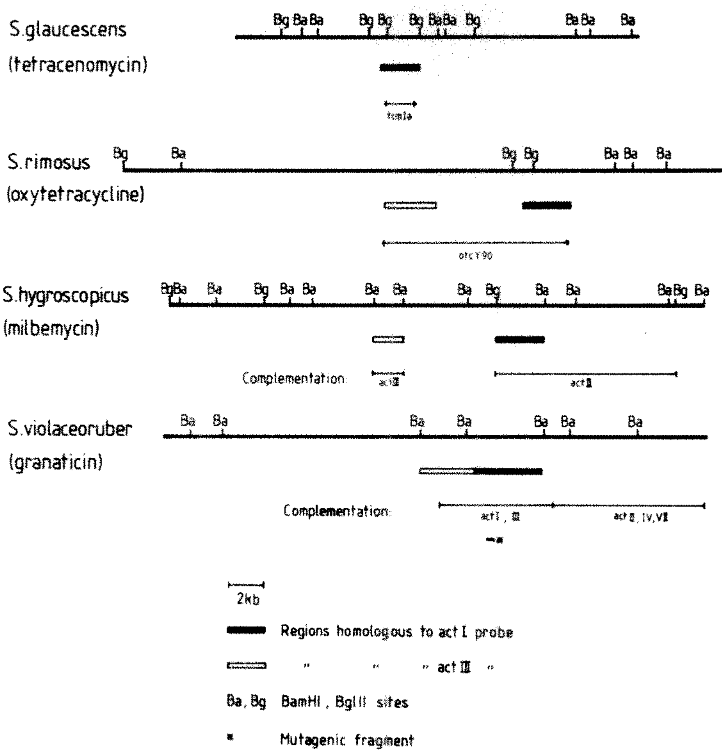

Fig. 3 Restriction maps of cloned DNA involved in polyketide synthesis from four different streptomycetes. The *S. glaucescens tcm* DNA and *S. rimosus otc* DNA segments were cloned and mapped previously[8,9]. *S. hygroscopicus* DNA was isolated in the present work by probing a colony library in *Escherichia coli* cosmid pIJ680 (T. Kieser, personal communication) with a purified *act*III fragment (*Bam* HI sites 13-14: ref. 5); *S. violaceoruber* DNA was isolated by probing a λ EMBL4[15] plaque library with pIJ2338, which is pBR329 carrying *act*I + *act*III DNA (*Bam*HI sites 13-17: ref. 5). Regions of the four DNA segments homologous with *act*I and *act*III from *S. coelicolor* are indicated. The abilities of certain DNA segments to complement blocked mutants of the same strain (*tcm*Ia or *otc*Y90) or various classes of *act* mutants of *S. coelicolor*, when introduced on pIJ941 or pIJ943[16], are also shown. The bar marked with an asterisk shows the fragment of *S. violaceoruber* DNA capable of generating granaticin non-producing lysogens when cloned into ϕC31 KC516[17], thus demonstrating that the cloned DNA is part of a set of biosynthetic genes for granaticin. For *S. hygroscopicus* the 1.6-kb *Bam*HI fragment homologous to *act*III, when cloned into ϕC31 derivative pPOD9 (C.R.B., unpublished), gave rise to defective lysogens which failed to release phages. The lysogens were milbemycin non-producing and Southern hybridization showed them to have deletions of DNA from the chromosomal region surrounding the cloned *Bam*HI fragment. This suggests that the cloned DNA contains genes involved in milbemycin biosynthesis.

(Fig. 1). This region of the cloned *S. glaucescens* DNA complements *tcm* mutants of class Ia, which probably lie in the tetracenomycin polyketide synthase gene[8]. A reciprocal experiment in which the *tcm* DNA was used as a probe against *act* DNA showed homology only to the *act*I region. Interestingly, there was no homology between the *act*III probe and the *tcm* gene cluster. We return to this point later.

Similar experiments identified regions with weak homology to either *act*I or *act*III in cloned *S. rimosus* DNA[9] carrying genes (*otc*) for oxytetracycline biosynthesis (Fig. 3). DNA from the homologous region complements *otcY90*, a mutation blocking oxytetracycline biosynthesis at one of the earliest steps[10]. Homology between the *act* probes and the cloned *otc* DNA was much weaker than with the *tcm* DNA, correlating with the failure to observe hybridizing bands in genomic digests of *S. rimosus* (Fig. 1).

Next we attempted to isolate genes for polyketide synthesis from the milbemycin-producing *S. hygroscopicus* ssp. *aureolacrimosus* and the granaticin-producing *S. violaceoruber* Tü22 by probing clone libraries with *act*I and *act*III DNA. In both species, hybridizing clones were found. Restriction mapping and further hybridizations identified linked sequences homologous to *act*I and *act*III (Fig. 3) on restriction fragments corresponding to those seen in genomic digests (Fig. 1). Functional homology of the cloned genes with those in the actinorhodin pathway of *S. coelicolor* was demonstrated by introducing the cloned *S. hygroscopicus* or *S. violaceoruber* DNA homologous to *act*III into an *act*III mutant of *S. coelicolor*, whereupon pigments similar or identical to actinorhodin were produced. Other classes of *act* mutants were complemented by different segments of the cloned *S. hygroscopicus* DNA (*act*II) or *S. violaceoruber* DNA (*act*I, II, IV, VII), indicating that groups of genes from polyketide biosynthetic pathways had indeed been cloned (Fig. 3). To confirm that these genes were involved in the granaticin or milbemycin biosynthetic pathways, the technique of mutational cloning was employed. In this approach mutations are generated when an *att*-site-deleted ϕC31 phage vector forms a lysogen by integration into the host genome through recombination between a cloned DNA segment internal to a transcription unit and homologous DNA in the recipient[11]. Granaticin non-producing or milbemycin non-producing lysogens were obtained with the test clones, indicating that they carried genes involved in the biosynthesis of these antibiotics (see Fig. 3 legend).

It is significant that DNA homologous to both *act*I and *act*III is found in the oxytetracycline, granaticin and milbemycin gene clusters, but only to *act*I in the tetracenomycin DNA (Fig. 3). Unlike the other four polyketides, tetracenomycin requires no reductive step associated with carbon chain assembly. If *act*III is indeed a reductase gene (see above), homology to *act*III would therefore not be expected in the tetracenomycin gene cluster.

The carbon chains of four of the antibiotics—actinorhodin, granaticin, tetracenomycin and oxytetracycline—are derived by condensation of acetate-derived residues, giving chains of varying length; for milbemycin, acetate- and propionate-derived residues must be condensed in the correct sequence. Moreover, as shown in Fig. 1, there is circumstantial evidence for homology between the *act* probes and DNA involved in the biosynthesis of other classes of antibiotics, including macrolides and polyethers with further patterns of chain assembly. Thus the cloning strategy demonstrated here is likely to be widely applicable. It might be extended by the use of probes to recognize cryptic producers of secondary metabolites of a particular class. Thus, *S. parvulus*, one of the apparent polyketide non-producers that contained DNA sequences hybridizing with the *act*I probe (Fig. 1), was recently found to have a normally unexpressed capacity for polyketide biosynthesis, producing the polyether nonactin when its ability to make the non-polyketide actinomycin was blocked by mutation (U. Keller, personal communication). The principles established here for polyketides could probably be extended to other chemical classes of antibiotics such as β-lactams, aminoglycosides or glycopeptides.

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Poly(dA)·poly(dT) is a B-type double helix with a distinctively narrow minor groove

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The structure of poly(dA)·poly(dT) currently arouses great interest, mainly because $dA_n \cdot dT_n$ stretches are associated with considerable DNA bending[1,2]. Until recently the heteronomous DNA described by Arnott *et al.*[3], with the poly(dA) and poly(dT) chains in A and B conformations respectively, was the only detailed model of this structure. Following our earlier studies of the interaction of DNA and monovalent ions[4–6], we examined the X-ray diffraction of the bivalent Ca^{2+} salt of poly(dA)·poly(dT) (Ca-poly(dA)·poly(dT)) and found no sign of a heteronomous structure: Ca-poly(dA)·poly(dT) in fibres shows fully equivalent B-type conformations of the opposite sugar–phosphate chains. A revision of the structure of the sodium salt, Na-poly(dA)·poly(dT), based on this result, yields only a slightly heteronomous structure with each chain in a B-type conformation, which is in much better agreement with the experimental data underlying the original heteronomous model[3]. Both structures, Ca- and Na-poly(dA)·poly(dT), have a minor groove narrower than that of the B form: this peculiarity seems to be very important for the interaction of poly(dA)·poly(dT) and biologically significant molecules (including proteins and antibiotics). The specific base-pair positions in poly(dA)·poly(dT) may account for the DNA bending adjacent to $dA_n \cdot dT_n$ tracts.

Figure 1 shows the diffraction pattern of Ca-poly(dA)·poly(dT). Some important features of the crystal and molecular structure were revealed by comprehensive Patterson analysis.

Figure 2*a* and *b* shows cylindrically averaged Patterson functions for Ca-poly(dA)·poly(dT) and for Li-B-DNA, for which a more reliable structure has been obtained[3,7]. These functions point to the similarity of the molecular structures of Li-B-DNA and Ca-poly(dA)·poly(dT) and to the fact that in the Ca-poly(dA)·poly(dT) unit cell, as in the classic crystalline B form, two molecules are related by translation. The systematic reflection absences in the *h k* 0 plane and the Patterson projection for the 0 *k l* plane also point to translation. We may conclude that, the T–A and A–T base pairs being indistinguishable (at the existing resolution of about 0.3 nm), the presence of the translation relation indicates a dyadic relation between the conformations of the poly(dA) and poly(dT) chains of a molecule (see Fig. 2 legend). Poly(dG)·poly(dC) in the A form[8] is a most convincing example of such a situation, where the molecular dyad is also a crystallographic symmetry element.

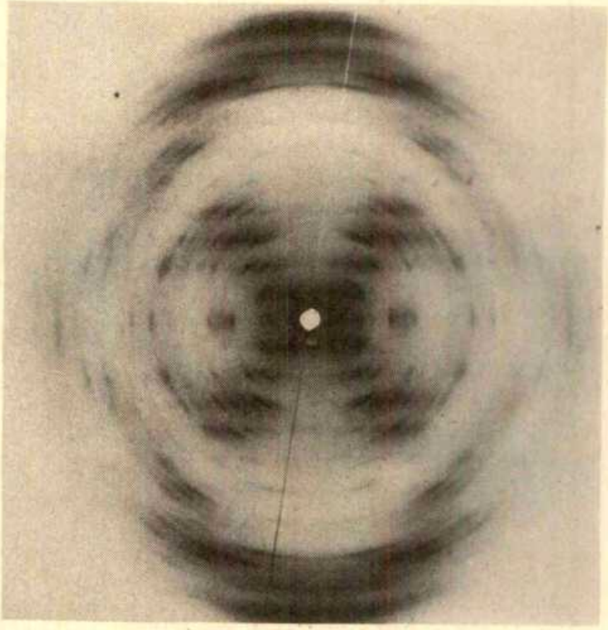

Fig. 1 Diffraction pattern of Ca-poly(dA)·poly(dT) (81% r.h.) in which the intensities of 87 independent diffraction spots were measured. Analysis of this pattern shows an orthorhombic lattice; the unit cell ($a = 1.871$ nm, $b = 4.032$ nm, $c = 3.232$ nm, $\alpha = \beta = \gamma = 90°$) contains two polynucleotide molecules. As in the case of the sodium salt[3], the pitch of the tenfold double helix is 3.23 nm and the molecules are packed into the same kind of flat layers in the *a* direction, although in our case the distance between them (in the *b* direction) is 0.48 nm larger. The structure was refined by the linked-atom technique (LALS program[9]), considerably modified in our case. We used a one-parameter flexible sugar ring based on the statistical analysis of the nucleoside and nucleotide structures from the Cambridge database[30]. Compared to ref. 3, we did not introduce any additional parameters defining the position of bases. The R value ($R = \sum |F_{obs} - F_{calc}| / \sum F_{obs}$) for the refined structure of Ca-poly(dA)·poly(dT) is 0.31, and $R'' = (\sum (F_{obs} - F_{calc})^2 / \sum F_{obs}^2)^{1/2} = 0.36$. R'' was calculated taking into account unobserved reflections in those cases where the calculated amplitudes exceeded the estimated threshold. Water correction has been made according to ref. 7.

As a result the heteronomous model[3] proves unacceptable for Ca-poly(dA)·poly(dT), for it must lead to the disappearance of translation peaks corresponding to the position of the second molecule in the unit cell (Fig. 2*a*). Then the only possible space group (as in the case of Li-B-DNA) is $P2_12_12_1$. A three-dimensional Patterson synthesis independently confirms the presence of translation and provides an image of the polynucleotide molecule (Fig. 2*c*). As a result of this procedure we have obtained the azimuth orientation of the molecule in the unit cell and a fairly good approximation of the phosphate group positions.

The sugar conformations in the refined Ca-poly(dA)·poly(dT) structure are close to C2′-*endo.* The search for models with different conformations for poly(dA) and poly(dT) chains has shown the Ca-poly(dA)·poly(dT) structure to lack heteronomy within the accuracy of the linked-atom technique (LALS method)[9].

Because the packing and molecular parameters of Na-poly(dA)·poly(dT) are close to those of the calcium salt, we attempted to adapt our model of Ca-poly(dA)·poly(dT) to the X-ray diffraction data from ref. 3. The result is a Na-poly(dA)·poly(dT) structure in which the poly(dA) and poly(dT) chain conformations differ much less than in the heteronomous model and the whole structure is fairly close to Ca-poly(dA)·poly(dT). The newly obtained structure of Na-poly(dA)·poly(dT) is free of overshort contacts of non-bonded atoms (intramolecular or intermolecular) and has an R value of 0.26 ($R'' = 0.29$). Under the same calculation conditions the

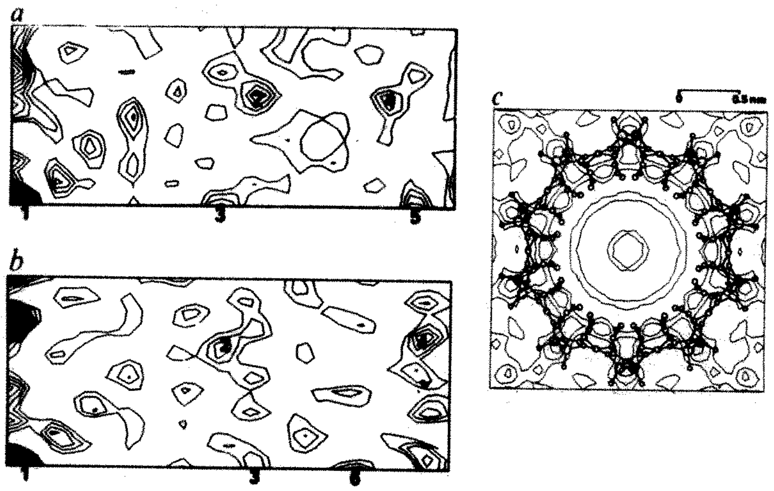

Fig. 2 *a,b*, Cylindrically averaged Patterson functions for (*a*) Ca-poly(dA)·poly(dT) and (*b*) Li-B-DNA. The vertical size of the figures corresponds to a half-length of the c side of the unit cell: 1.616 nm for *a* and 1.690 nm for *b*. The peak of the function with coordinates $r = 0$ and $z = 0$ (number 1) corresponds to zero interatomic vectors. The DNA double helix diameter is ~2 nm, as intramolecular peaks are located in the $r < 2$ nm region and only intermolecular peaks are located in the $r > 2$ nm region. In the case of Li-B-DNA[7] we know the positions of the two molecules in the unit cell, so that we can find the Patterson function peaks corresponding to all intermolecular translations. Thus, peaks 3 and 6 correspond to lattice translations (along sides b and a[7]). Peaks 2 and 4 correspond to non-lattice translations relating the two molecules in the unit cell, which are shifted relative to each other by $\sim c/3$ (ref. 7) along the z axis. The nucleotide sequence is random in the case of Li-B-DNA, which makes such translations obvious, but in the case of Ca-poly(dA)·poly(dT) there is also a complete set of intermolecular translation peaks similar to those of Li-B-DNA. As it is highly unlikely that the long poly(dA)·poly(dT) molecules would form crystallites with all molecules directed one way, the translation peaks must indicate the presence of traditional molecular dyads running perpendicular to the helix axis. Here peaks 3 and 5 correspond to lattice translations, which are equal to $|a|$ and $|2a|$, and peaks 2 and 4 correspond (as with Li-B-DNA) to non-lattice translations, the relative shift (Δz) of the two molecules in the unit cell being $\sim c/3$. *c*, Electron density square for Ca-poly(dA)·poly(dT) molecule (minus the constant), calculated according to the three-dimensional Patterson synthesis (within the framework of space group $P2_12_12_1$). The projection is made down the helix axis. The basis of this approach is similar to that of the previously described 'helical projection' derived from a cylindrically averaged Patterson function[31]. The squared electron density maxima are in good agreement with the superimposed sugar–phosphate chains of the refined model. The radii of phosphorus atoms are shown (large circles) reduced 5 times compared to their van der Waals radii; the other atomic sizes are reduced 8 times.

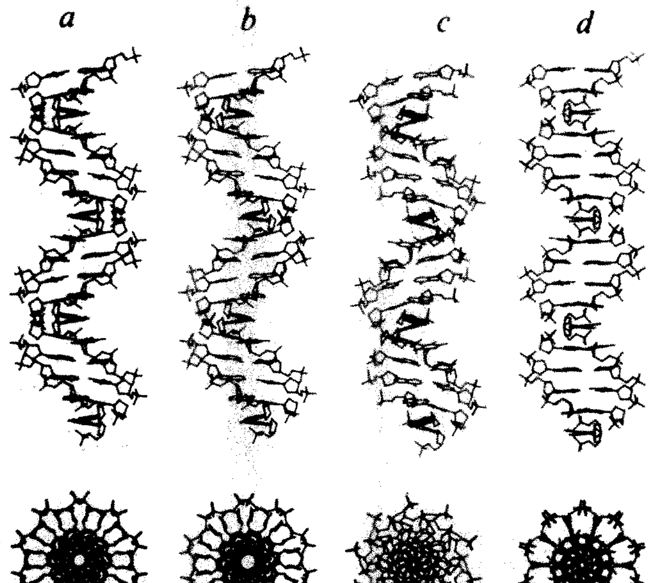

Fig. 3 Projections perpendicular to (above) and down (below) the helix axis for: *a*, the calcium salt; *b*, the sodium salt of poly(dA)·poly(dT); *c*, the heteronomous model of Na-poly(dA)·poly (dT)[3]; *d*, poly(dA)·poly(dT) in the classic B form[3]. The width of the minor groove in these structures (the shortest distance between the phosphorus atoms of opposite chains) is 0.92 nm, 0.92 nm, 1.1 nm and 1.2 nm respectively. The conformation at 03′ – P is *gauche minus* in our models of poly(dA)·poly(dT) and not *trans* as in the B-DNA[3]. This is in agreement with the crystal data[11]. The bases are shifted about 0.1 nm away from the helix axis (in the same direction as in A-DNA). The shift and tilt of −6° account for the reduced pitch in the poly(dA)·poly(dT) double helix (by 0.15 nm) compared with the B form[7]. The standard deviation for the backbone torsion angles between poly(dA) and poly(dT) chains is 10° in our Na-poly(dA)·poly(dT) model, whereas in the heteronomous model[3] it is 45°. In our case the effective relative shift (Δz) of the two counter-directed Na-poly(dA)·poly(dT) molecules in the unit cell (space group $P2_1$) is close to zero, and the azimuth orientation of the molecules is close to that in Ca-poly(dA)·poly(dT), where one of the pseudodyads is directed along the b side. The situation is essentially different in the packing of heteronomous molecules (S. Arnott, personal communication), although in both cases the molecules are situated at the vertices of a 72° rhombus. A full set of molecular and crystal packing parameters will be given elsewhere.

heteronomous model has an R value of 0.28 ($R'' = 0.37$) even though it was developed with a greater number of parameters[3], and so can be rejected with 99.5% confidence according to Hamilton's statistical significance test[10]. Figure 3 shows the optimized structures of Ca- and Na-poly(dA)·poly(dT). The observed base positions (the negative tilt of −6° and the large positive propeller twist of 20°) in both structures considerably narrow down the minor groove of the double helix (by 0.28 nm compared with the classic B form).

These new structures show a striking similarity to the A·T stretch of the CGCGAATTCGCG dodecamer in crystals[11,12] (for example, the shortest distances between phosphates virtually coincide with those in Fig. 3). One of the important features of the dodecamer is the double-layer hydration spine in the minor groove of the A·T stretch, which stabilizes the B-type structure[12,13]. An earlier study of Cs-B-DNA fibres[5] revealed an ionic stabilization mechanism of the B form which is similar to the water mechanism. Model estimations of the sodium ion positions in the newly found structure of Na-poly(dA)·poly(dT) have shown that only the latter mechanism is possible (see Fig. 4). A double-layer water spine should be present inside the minor groove in the case of Ca-poly(dA)·poly(dT) as well, because the Ca^{2+} ions that could, in principle, be situated there, are in reality located near the phosphate groups, as attested by the results of extended X-ray absorption fine structure (EXAFS) spectroscopy[14,15].

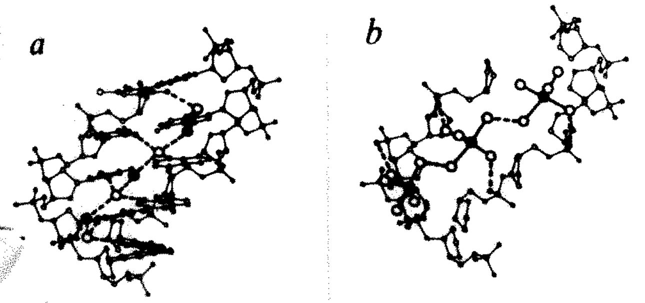

Fig. 4 *a*, The double-layer water spine in the minor groove of poly(dA)·poly(dT) similar to that observed in the CGCGAATTCGCG dodecamer[12,13]. Five successive nucleotide pairs are shown. Dashed lines denote hydrogen bonds. Second layer water molecules are crossed. According to our previous calculations for the poly(dA)·poly(dT) molecule in the B form, Na^+ ions, like Cs^+ ions[5] or water molecules of the first layer, can enter into close coordination with N3 atoms of adenines and O2 atoms of thymines (see Figure of ref. 4). In the newly determined structure of Na-poly(dA)·poly(dT), such a model is at variance with the narrowed minor groove: very strong distortions occur in the $(O2/N3)\text{-}Na^+\text{-}(H_2O)_4$ octahedron. By contrast, the octahedral coordination of Na^+ can be kept practically undistorted if the ions are positioned in the third solvent layer adjacent to the double-layer water spine. *b*, A model of the integration of Na^+ ions into the poly(dA)·poly(dT) structure on the side of the minor groove. The base atoms and the double-layer water spine are not shown. Ions (black circles) are situated over the second-layer water molecules (see *a*) and interact with them. Optimized sodium-water octahedrons are linked by hydrogen bonds both to each other and to the phosphate oxygens of the opposite chains in the double helix. Such an ion-water spine meets with no steric obstacles in the crystal lattice.

These conclusions are supported by the energy calculations of Chuprina[16-18], which suggest that a water spine in the minor groove of poly(dA)·poly(dT) would lead to an energetically optimal structure with the distinctive features we observe in fibres. It seems quite likely that the water spine makes a substantial contribution to the stabilization of the poly(dA)·poly(dT) structure and is largely responsible for its marked conservatism in different circumstances. The molecular structures of Ca- and Na-poly(dA)·poly(dT) are similar; they do not adopt the A form; moreover they retain the B′ structure, virtually unchanged, at any relative humidity. This polynucleotide does not adopt the A form in water-alcohol solutions either[19]. Furthermore, even short (4 base-pair) $dA_n\cdot dT_n$ insertions in a quasi-random DNA sequence retain the fibre-characteristic helical periodicity of 10.0 base pairs per turn in solution[20,21], whereas for other sequences in solution this quantity changes to 10.6 ± 0.1[20-22].

Our results support the concept that the junctions between this conservative structure and the B form are responsible for the equilibrium bends in some natural DNA fragments[1,23,24], for the equilibrium base positions in our structures of Ca- and Na-poly(dA)·poly(dT) differ from their positions in the B form. The proposed direction of bending[1] is in good agreement with structural findings presented here. The observed poly(dA)·poly(dT) structure in fibres also testifies in favour of the narrowed minor groove in A·T sequences. Experiments involving the cleavage of DNA by some nucleases[25,26], the binding of non-intercalating drugs such as netropsin[27,28] or the binding of some minor-groove recognition proteins[29] suggests that the distinctive narrowness of the minor groove may have specific biological significance.

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Long-range cooperativity between gene regulatory sequences in a prokaryote

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Regulation of transcription initiation by proteins binding at DNA sequences some distance from the promoter region itself seems to be a general phenomenon in both eukaryotes and prokaryotes. Proteins bound to an enhancer site in eukaryotes can turn on a distant gene, whereas efficient repression of some prokaryotic genes such as the *gal*, *ara* and *deo* operons of *Escherichia coli*, requires the presence of two operator sites, separated by 110, 200 and 600 base pairs (bp) respectively[1-6]. In the *deo* operon, which encodes nucleoside catabolizing enzymes, we have shown that efficient and cooperative repression can be obtained when the distance between the two sites ranges from 224 to 997 bp[5,6]. Here, we report that transcription initiation can be regulated from an operator site placed 1 to 5 kilobases (kb) downstream of the *deoP2* promoter (and downstream of the transcribed gene), and present the first experimental data for prokaryotic regulation at distances >1 kb. Our results support the model of DNA loop formation as a common regulatory mechanism explaining both some prokaryotic regulation and the action of eukaryotic enhancers[7].

Full *deoR* repression of the *deoP1* and *deoP2* promoters requires the presence of three *deoR* binding sites[5,6]. Two of these operators, 599 bp apart, overlap the sites of transcription initiation (*deoP1* and *deoP2*, Fig. 1*a*) whereas the third site O_E is located 279 bp upstream of *deoP1*[5,6,8]. The *deoP1* and *deoP2* operator sites alone are sufficient to obtain a *deoR* regulation

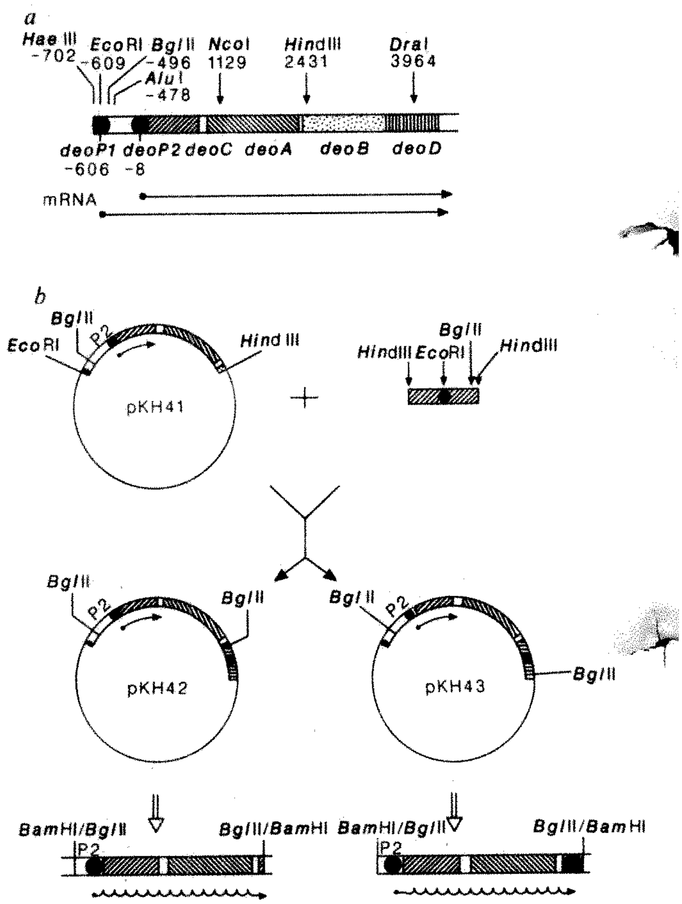

Fig. 1 *a*, Structure of the *deo* operon. The *deo* genes code for deoxy-riboaldolase (*deoC*), thymidine phosphorylase (*deoA*), phosphopentomutase (*deoB*) and purine nucleoside phosphorylase (*deoD*). Tetracistronic transcripts are initiated from two promoters *deoP1* and *deoP2* which each contain a *deoR*-binding site (black circle) overlapping the −10 region of the promoter (refs 5, 8; ref. 12 for review). The third *deoR* binding site O_E located 279 bp upstream of *deoP1*[6], is not shown here as it is not present in any of the plasmids used in this work. Restriction sites are indicated and numbered relative to the start of mRNA from *deoP2*. *b*, Construction of plasmids used in Fig. 3. The vector used is the mini R1 plasmid pJEL126. This 19.5-kb plasmid is present at only one copy per genome, and contains unique *Eco*RI and *Bam*HI sites proximal to *lacZ*[6,9]. A 3,040-bp *Eco*RI–*Hin*dIII fragment containing the *deoP2-deoC-deoA* operon was isolated from pVH19 (P.V.-H., in preparation) and inserted into a pBR322 derivative (pGD3) resulting in pKH41. This plasmid contains unique *Bgl*II and *Hin*dIII sites on each side of the *deoP2-deoC-deoA* operon. A 224-bp *Hae*III–*Alu*I fragment containing the *deoP1* operator[5] was inserted into the *Hin*dIII site of pKH41 after ligation of a *Hin*dIII linker (pKH42 and pKH43). By transferring the *Bgl*II fragment from these plasmids into the *Bam*HI site of the low-copy-number plasmid pJEL126[6], two plasmids were constructed: pGD108 contains the *deoP2* promoter/operator followed by the *deoC* and *deoA* genes and the *deoP1* operator placed 2,540 bp downstream of the *deoP2* operator. The other plasmid pGD103 contains only the *deoP2* operator. Plasmids pKH162, pKH163 and pKH165 (see Fig. 3) were derived from pKH41 by a similar procedure, pKH41 first being digested with *Nco*I, the sticky ends filled in with DNA polymerase (Klenow fragment) and a 12-bp *Hin*dIII linker ligated to the blunt ends (pGD35). This new *Hin*dIII site was used for insertion of the *deoP1* operator and the entire construction was transferred to pJEL126 on a *Bgl*II fragment as above. In pKH165 a *deoP1* operator fragment was used in which the operator had been destroyed by filling in the *Eco*RI site in the centre of the *deoR* binding site[5]. To construct pGD110 and pGD107 the *Bgl*II fragment of pKH42 and pKH43 was first inserted into pGD201, a pBR322 derivative containing a unique *Bgl*II site and no *Hin*dIII sites. In the resulting plasmids pGD203 and pGD204 the *Hin*dIII site (position 2,431) of the *deo* operon is now unique and the entire *deo* operon was thereafter reconstructed by inserting a *Hin*dIII–*Dra*I fragment (2,431–3,964). This DNA fragment was isolated from pVH7 (P.V.-H., in preparation) and a *Hin*dIII linker was ligated to the *Dra*I end. The *Bgl*II fragment of each of the resulting plasmids (pGD205 and pGD206) was inserted into the *Bam*HI site of pJEL126 as described above giving pGD110 and pGD107 respectively.

of 20–30-fold compared with 2-fold when only one of the promoter/operator sites is present[5,6]. In this study we asked first whether it is possible to obtain regulation of transcription initiation from an operator site positioned downstream of the transcribed region, and second, if so, to what extent repression depends on the distance between the two operator sites.

First, we used low-copy-number plasmids encoding *deoP2* gene fusions between *deoC* or *deoA* and *lacZ* (refs 6 and 9; Fig. 2, lines *a* and *d*). When the *lacZ* fusion is transcribed from *deoP2* containing the *deoP2* operator site alone, the *deoR* regulation is 1.8-fold (lines *a* and *d*). When a second *deoR* binding site is placed 567 bp upstream of the *deoP2* operator a drastic decrease in the wild-type level is observed resulting in a 23-fold regulation by *deoR* (line *b*). Next we inserted the second operator site downstream of the *lacZ* gene in the *deo-lacZ* fusions, which resulted in a small but significant increase in the *deoR* regulation (lines *c* and *e* compared with lines *a* and *d*). The distance between the operator sites in these two constructions is 3,702 and 4,685 bp and the *deoR* regulation is 3.6- and 3.0-fold respectively.

To determine in more detail how repression depends on the distance between the two operator sites, we used low-copy number vectors containing the *deoP2* promoter and varying parts of the *deo* operon (Figs 1 and 3). Expression of the first structural gene, *deoC*, from these plasmids was determined in both wild-type and *deoR* cells (Fig. 3). In accordance with the results in Fig. 2, transcription initiation from *deoP2* is regulated weakly by the *deoR* repressor (1.6–2-fold) when only one *deoR* binding site is present on the plasmid. Insertion of a second *deoR* binding site downstream of the *deoP2* operator increases the *deoR* repression in all three experiments shown in Fig. 3. The distance between the operator sites was 1,245, 2,540 and 4,076 bp and repression was 21-, 8.8- and 5.2-fold, respectively. This inverse relationship between the extent of repression and the distance separating the two operator sites is evident when the data from Fig. 3 are used in a double-log plot, where a straight line is observed (data not shown). Extrapolation of this line to <1 kb is probably not meaningful because the resistance of the DNA molecule to bending and twisting over such short distances is high[10,11]. Extrapolation towards long distances indicates that a twofold repression should be obtained in these fusions when the two operators are separated by 8–10 kb. A twofold *deoR* repression corresponds to that observed for *deoP2* alone, and thus, the cooperativity between the two *deoR* binding sites in our system is expected to be lost at 8–10 kb. We have tested a distance of 39 kb by using an *E. coli* strain containing two λ phages inserted in tandem each carrying a *deoP1-galK* fusion with only one *deoR* operator. As expected, no increase in *deoR* repression was observed (data not shown).

We have included three different controls in Fig. 3. First, insertion of a mutated *deoR* binding site downstream of the *deoP2* operator results in the same low degree of repression as that observed for the *deoP2* plasmid with no insert (pKH165 compared with pKH162). The additional repression observed when two operator sites are present on the same plasmid is therefore due to the presence of the second *deoR* binding site. Second, the twofold repression seen in the three constructions containing only one *deoR* binding site shows that the *deo* operon does not contain any *deoR* binding sites between *deoP2* and *deoD* (pKH162, pGD103 and pGD110; see also Fig. 2, lines *a*

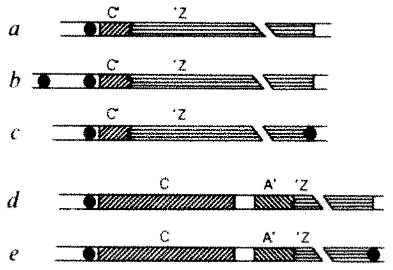

Fig. 2 Effects of a second *deoR* operator site on *lacZ* expression from *deoP2-lacZ* fusions in the presence and absence of *deoR* repressor. Left, constructions as follows: *a*, pJEL190; *b*, pJEL190-O$_1$A; *c*, pJEL190-O$_1$B; *d*, pJEL194; and *e*, pJEL194-O$_1$B. C and A, *deoA* gene and *deoC* gene (diagonal hatching); Z, *lacZ* gene (horizontal hatching); ', side of gene deleted; ●, operator sites. Right, distance between operators and *lacZ* expression for each plasmid, in wild-type (wt) and *deoR* background. Repression is given as the ratio of expression in *deoR* to that in wild-type.
Methods. The *deo-lacZ* protein fusions pJEL190 (*a*) and pJEL194 (*d*)[6] contain a unique *Asu*II restriction site in the *lacY* gene and two *Cla*I sites, one in the *lacZ* gene and the other in *deo* DNA, 555 bp upstream of the start point of *deoP2* transcription[8]. A purified *Cla*I fragment from pVH17[8] containing *deo* sequences 21 bp upstream to 54 bp downstream of the central base pair in the *deoP1* operator was inserted into pJEL190 partially digested with *Cla*I and into the unique *Asu*II site of pJEL190 and pJEL194, resulting in plasmids pJEL190-O$_1$A, pJEL190-O$_1$B and pJEL194-O$_1$B. Measurements of β-galactosidase expressed from fusion plasmids were done as described by Miller[16]. Samples for enzyme assays were taken at OD$_{450}$ in the range 0.1–0.5. Specific activities of β-galactosidase are expresssed as OD$_{420}$/OD$_{450}$ ml^{-1} min^{-1}. Cells were grown exponentially at 36 °C in AB medium[17] with glycerol as carbon source. All experiments were performed with the *E. coli* K-12 strains Sϕ928 (Δ*deo*, Δ*lac*) and Sϕ930 (Δ*deo*, Δ*lac*, *deoR*)[18].

	lacZ		
Distance (bp)	wt	*deoR*	Repression
–	0.5	0.9	1.8
567	0.06	1.4	23.0
3702	0.22	0.8	3.6
–	0.65	1.2	1.8
4685	0.44	1.3	3.0

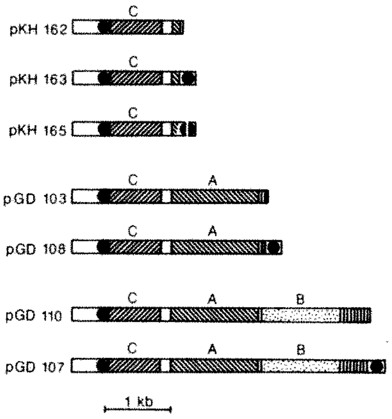

Fig. 3 Effects of a second operator site on *deoP2* expression of the *deo* operon. Left, plasmid constructions containing *deo* genes. C, A, B: *deoC*, *deoA*, *deoB* genes respectively; ●, operator sites; vertical hatching, parts of the *deoD* gene. Right, distance between operators and *deoC* and *deoA* expression in wild-type (wt) and *deoR* backgrounds. Repression is given as the ratio of expression in *deoR* to that in wild-type.
Methods. The second operator site was inserted into the *Nco*I, *Hin*dIII or *Dra*I sites as in Fig. 1. The activity of plasmid-encoded deoxyriboaldolase (*deoC*) and thymidine phosphorylase (*deoA*) was measured in extracts of Sϕ928 and Sϕ930 harbouring the plasmids. Cells containing the recombinant low-copy-number plasmids were grown exponentially at 33 °C for 5 generations in AB medium[17] supplemented with 25 μg ml^{-1} ampicillin and 0.2% glycerol as carbon source. At a cell density of 1.5×10^8, cells were collected by centrifugation, washed in ½ vol. AB and finally resuspended in 1/60 vol. 100 mM Tris-HCl *p*H 7.6, 2 mM EDTA, and sonicated for 60 s. The supernatant was used for the determination of deoxyriboaldolase and thymidine phosphorylase as described[19,20]. Units are expressed as nmol of substrate converted per min at 37 °C. The numbers given are specific activities, expressed as units of enzyme activity per mg of protein determined according to Lowry *et al.*[21]. Numbers represent the mean of at least three independent experiments. We used the low-copy plasmids where *deoP2* transcription reads in the opposite direction to that of the *lacZ* gene. Quantitatively similar results were obtained with the constructions where *deoP2* reads into *lacZ*, but in these plasmids the basal level is slightly elevated due to an unknown plasmid promoter activity (data not shown).

	deoC			*deoA*		
Distance (bp)	wt	*deoR*	Repression	wt	*deoR*	Repression
–	191	389	2.0		–	
1245	17	357	21.0		–	
–	210	361	1.7		–	
–	217	422	1.9	195	340	1.7
2540	49	427	8.8	43	282	6.6
–	281	444	1.6	588	954	1.6
4076	85	444	5.2	216	997	4.6

and *d*). Third, when the activity of the *deoC* and *deoA* gene products was measured for the four plasmids carrying both genes, the *deoR* regulation was found to be the same (Fig. 3).

The main conclusion from the results in Figs 2 and 3 is that it is possible to construct an operon in *E. coli* where transcription initiation is regulated from an operator site at a position downstream of the transcribed genes. The degree of repression is inversely related to the distance between the operator sites and can be observed even at a distance of 4.7 kb.

The cooperativity between the *deoR* binding sites is the same whether they are present on a low-copy-number plasmid (mini R1)[6] or on the bacterial chromosome (λ phage)[5]. One requirement is, however, that the binding sites are present on the same DNA molecule because the additional *deoR* binding sites carried on the bacterial chromosome at *nupG* and *tsx*[12] have no effect on the repression in our experiments. We have observed cooperativity at the following distances: 224, 279, 449, 567, 599, 603, 798, 878, 997, 1,245, 2,540, 3,702, 4,076 and 4,685 bp (see also refs 5 and 6). The DNA loop model[2,3,5] can be used to explain all our data. According to this model, in the repressed state the same *deoR* repressor complex is bound to both operator sites joined together through the DNA loop. The increased stability of such a complex is analogous to that of a metal-chelate complex, where the metal binding (*deoR* repressor) is increased because it is held by at least two 'arms' of the chelater (DNA). The model requires the formation of a repressor-protein complex containing two operator binding sites, and a DNA molecule with binding sites which are physically close to each other in the cell.

Distances ranging from 1 to 4 kb have also been used in DNA-ligation studies in which the kinetics of cyclization and of bimolecular reactions were compared[10]. The results showed an increase in the effective concentration of sticky ends when they were present on the same molecule. Furthermore, with increasing distance between the ends, this effective concentration decreased in a way very similar to the results presented here.

The formation of protein-DNA loops seems to be a common mechanism for transcriptional regulation in both prokaryotes and eukaryotes[2,3,5-7,13-15]. The immediate function of the loop seems to be to increase the local concentration of a particular protein in the promoter region resulting in repression (*deo* operon) or activation (enhancers). Regulation from a distance through loop formation is a very adaptable system allowing targets for the creation of binding sites for different regulatory proteins. This could probably facilitate the evolution of multiple control systems for promoter expression.

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Structure of co-crystals of tropomyosin and troponin

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Troponin, a Ca^{2+}-sensitive complex, regulates the motions of tropomyosin on the thin filaments in many muscles. It has three subunits, each with a different architecture and function: TnC binds Ca^{2+}; TnI binds to actin and inhibits contraction; and TnT binds one complex to each tropomyosin molecule[1,2]. The troponin complex has an elongated shape with TnC and TnI forming a globular 'head' region and TnT a long (~160 Å) 'tail'[3]. TnT binds to two widely separated regions of tropomyosin: the head region of the complex is near Cys 190 of tropomyosin and the tail region is near the overlapping joint that links the tropomyosin molecules into filaments[4]. Here we report the X-ray structure determination at 17 Å resolution of glutaraldehyde-treated tropomyosin crystals in which native troponin complex or fragments of TnT have been bound. Our results show that the amino-terminal tail end of TnT spans the head-to-tail joint of the tropomyosin filaments, and that the 'head' region of the whole troponin complex binds ~200 Å away near residues 150–180 of the tropomyosin molecule.

The Bailey crystal form is highly hydrated (95% solvent by volume) and its structure has now been solved to 15 Å resolution including visualization of the head-to-tail joint and correlation of the amino-acid sequence with the electron-density maps[5,6]. The open meshwork of this lattice (space group $P2_12_12$ with $a = 120$ Å, $b = 244$ Å and $c = 296$ Å) allows large molecules to be incorporated, but also means that the crystals are unusually labile. Thus the troponin complex can sometimes be co-crystallized in the lattice, but leads to large changes in unit cell parameters that have previously prevented determinations of its structure[7,8].

We find that treatment of pure tropomyosin crystals with 0.1% glutaraldhyde stabilizes the lattice yet allows the binding of troponin and its components. Diffraction patterns from these glutaraldehyde-treated tropomyosin crystals are virtually identical to those from untreated crystals. The glutaraldehyde reaction is completed before addition of other proteins, which are not crosslinked and bind in a specific manner. Using the structure of pure tropomyosin for initial phases, we have used solvent flattening techniques for phase refinement[5] including figure-of-merit weighting[9] of initial and solvent-flattened phases. Subsequent difference Fourier maps were used to locate the troponin in these crystals.

We have layered concentrated solutions of T1, the chymotryptic fragment of TnT of relative molecular mass 19,000 that comprise residues 1-158[10,11], in capillaries containing crystals of glutaraldehyde-treated tropomyosin, allowing the fragment to diffuse into the crystal and bind specifically to the filaments. Diffraction data have been collected by screened precession photography. The resulting structure has been solved using difference maps based on the solution of the native tropomyosin structure. The maps reveal the T1 fragment as an elongated mass ~95 Å long and a ≤35 Å in width situated on the cross-over regions in the lattice[6] and extending ~20-30 Å into the 'short arm' (Fig. 1). The fragment thus interacts with a considerable length of the carboxy-terminal end of tropomyosin and extends beyond the head-to-tail joint where it spans a short segment (10-15 amino acids) of the adjacent tropomyosin molecule.

Diffraction data have also been collected from uncross-linked co-crystals grown from a mixture of tropomyosin and the highly α-helical cyanogen bromide-digested CB2 fragment of TnT (residues 71-151)[12]. The unit-cell changes arising from the binding of this small fragment are minimal. Difference maps calculated using coefficients generated by subtracting the CB2 co-crystal data from the T1 co-crystal data reveal a distinct peak overlapping the head-to-tail joint of tropomyosin and extending up to the cross-over region of the lattice (Fig. 2). These results indicate that residues 1-70 of troponin T are involved in binding to the termini of tropomyosin and that residues 71-158 extend toward the middle of the tropomyosin molecule.

The entire troponin complex has also been diffused into the cross-linked tropomyosin lattice and visualized. The major features of difference maps calculated from troponin-tropomyosin crystal data minus native tropomyosin data show mainly the mass of the tail region of troponin and no peaks that would correspond to the head portion of the complex. Accordingly, phases of the structure factors for the troponin-tropomyosin crystals were refined using solvent flattening procedures[6]. Difference maps were then calculated using coefficients obtained by subtracting the T1-tropomyosin crystal data to reveal this head region (Fig. 3). The heads appear to be associated with residues 150-180 of tropomyosin, but are bound with lower occupancy than the tail region, and hence are more difficult to visualize.

Although glutaraldehyde was used to stabilize the tropomyosin crystal lattice, our evidence indicates tha the TnT binding sites we have identified correspond closely to those of the native molecule. Intensity data from one projection of co-crystals of the CB1 fragment of TnT (residues 1-151) and native tropomyosin (G.N.P. and C.C., unpublished data) were essentially identical to that from the T1-tropomyosin co-crystals. It is likely that only a few tropomyosin lysines react with glutaraldehyde, so that they key linkages holding the TnT to tropomyosin are largely unaffected.

These results define certain of the interactions between troponin and the filaments of tropomyosin. TnT binds along the tropomyosin molecule in an antiparallel fashion, with its amino terminus spanning the carboxy terminus of tropomyosin and a

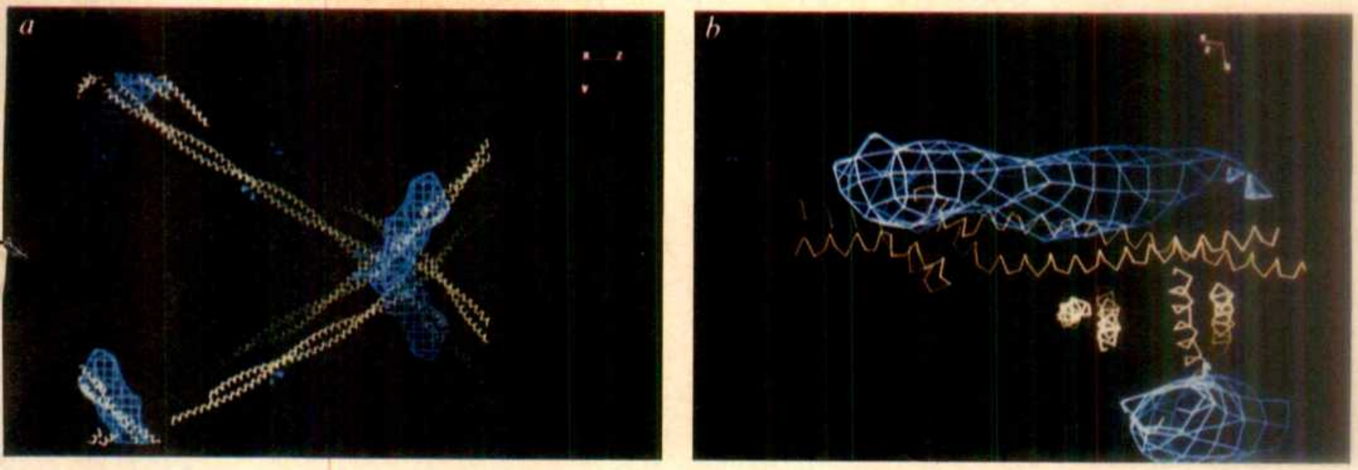

Fig. 1 Fourier map showing differences between tropmyosin-troponin T1 and pure tropomyosin crystals: *a*, view of the whole unit cell showing T1 fragments (blue) binding near the cross-over regions; *b*, different, enlarged view showing binding of T1 extending over the end-to-end joint of tropomyosin (a second symmetry-related T1 fragment is seen at bottom right). Superposed is a model of the α-carbon backbone of the coiled coil (yellow) built to follow the path of the molecule and including known stereochemistry of alpha helices.

Methods. Tropomyosin was purified as described previously[5] with the addition of hydroxylapatite chromatography as the final step. The crystals were grown in quartz capillary tubes at or near *p*H 5.8[23]. Troponin was prepared from rabbit skeletal muscle using a modified procedure of Greaser[1,2] and purified on a Whatman DE52 cellulose anion-exchange column eluted using a linear KCl gradient (adapted from ref. 24). The T1 fragment was prepared directly from troponin by digesting the latter with a 1/750 weight ratio of chymotrypsin (type alpha, Sigma) for 24 min on ice. The digest was fractionated on a Whatman DE52 column. Pure T1 was identified on SDS gel electrophoresis using standards provided by L. Smillie. A 50 mg ml^{-1} solution of T1 in crystallization buffer was prepared (100 mM sodium acetate, 50 mM ammonium sulphate, *p*H 5.8) and layered over an α-tropomyosin crystal pretreated with 0.1% glutaraldehyde. Glutaraldehyde pretreatment consisted of preparing a fresh solution of 0.1% glutaldehyde (Grade 1, Sigma) in crystallization buffer, layering this solution in the capillaries containing the crystals for 2-14 days at 4 °C. Data were collected by screened precession photography to a resolution of 17 Å (745 unique reflections). *R*-factors for merging the data from different film packs were 0.06-0.08. *R*-factors between native and CB2, T1 and Tn data were 0.10, 0.14 and 0.14, respectively. Phases for the difference maps were obtained by combining phases from the native tropomyosin structure[6] and phases from solvent-flattened maps of each of the troponin-tropomyosin co-crystals. Although the difference maps presented here were calculated using native data from crystals that had not been treated with glutaraldehyde, a control data set from glutaraldehyde-treated native crystals produced virtually identical results. This and subsequent images were calculated and displayed on a Silicon Graphics IRIS 2400 workstation using a modified form of FRODO software (ref. 25 and S. J. Oatley, unpublished data).

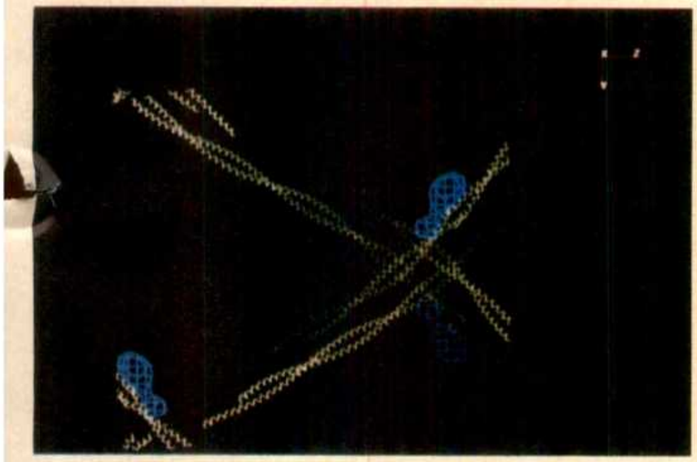

Fig. 2 Difference Fourier map between tropomyosin-troponin T1 and tropomyosin-troponin CB2 crystals. The additional mass present in the T1 crystals seen here reveals that residues 1-70 of TnT bind over the head-to-tail joint of tropomyosin.

small amino-terminal portion of the adjoining molecule. Previous biochemical studies indicated that residues 1-70 of TnT (CB3) interact with TnI and are not involved in binding near the head-to-tail joint of tropomyosin[13]. More recent evidence is consistent with the finding reported here of the interaction between CB3 and the head-to-tail joint[14]. TnT also extends ~100 Å toward Cys 190 of tropomyosin but shows little additional mass associated with tropomyosin residues 240-190. The head region of troponin, comprising TnI, TnC and perhaps a small portion of TnT, binds near residues 150-180 of tropomyosin.

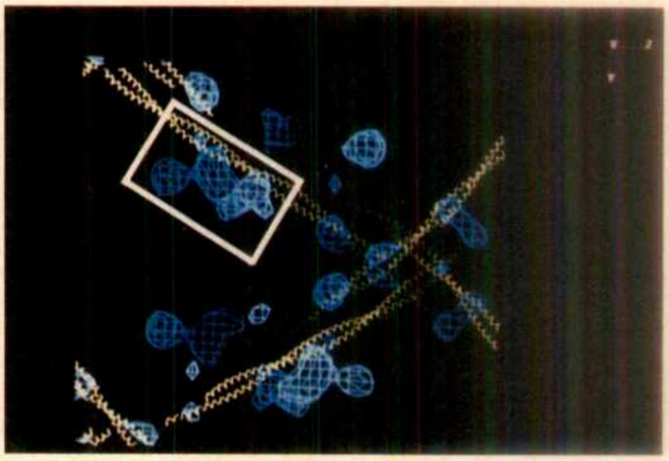

Fig. 3 Fourier map showing differences between tropomyosin-troponin and tropomyosin-troponin T1 crystals. Density corresponding to the head region of troponin is weak, but clearly visible. The white box encloses peaks corresponding to one troposin head. The upper left edge of the box intersects the tropomyosin molecule at residue 120, and the lower right edge at residue 194. Proteins were purified as described in Fig. 1. A 100 mg ml^{-1} solution of troponin in 10 mM sodium acetate, 1 mM EGTA (ethylinibisoxyethylininitrilo tetraacetic acid), *p*H 5.8, was prepared and layered over a glutaraldhyde cross-linked tropomyosin crystal, which had been preincubated in the same buffer.

Our results suggest that part of the T1 structure is disordered in the crystal, with only the tropomyosin-binding portions visible in the difference maps. Thus T1 probably comprises about two-thirds of the 185-Å-long TnT subunit[3], but only about half the fragment can be seen. Such a picture also accounts for the lack of connectivity in the difference maps between the head and tail regions of troponin. This two-site model for the attachment of troponin to tropomyosin is in agreement with earlier biochemical studies of binding[4,15-17] and electron-microscope observations[3]. The head region of troponin appears to bind weakly to tropomyosin under the ionic conditions used here, and we are studying this interaction further.

Our results reported here together with other findings provide new information about the tropomyosin/troponin switch in muscle. A recent model for regulation suggests that it is primarily the troponin complex that holds tropomyosin extended along the actin helix in the 'off' or resting state[6]. We now suggest that troponin would be bound in a strong invariant link to tropomyosin only near the head-to-tail joint of the filaments. A highly α-helical portion of CB2, although not forming a regular three-chain structure[18], is likely to bind through electrostatic interactions to the two-chain coiled coil of tropomyosin. These interactions are not, however, in regions predicted previously by electrostatic considerations[19,20]. The middle portion of TnT might form a flexible link between the tightly bound tail region and the head of the troponin complex. The binding of the head region of troponin would depend on the Ca^{2+} saturation of TnC[16,17,21,22]: at low Ca^{2+} concentration, corresponding to the 'off' state of the switch, the linkages between the subunits are weak, whereas those between TnI and actin, and between TnT and tropomyosin, are relatively strong. In the presence of Ca^{2+}, the head of the troponin complex detaches allowing the movement of tropomyosin to a new position on the thin filament and the binding of force-producing myosin heads. This is the 'on' or 'potentiated' state of the switch (see also ref. 6). Whatever the Ca^{2+} level, the tail region of troponin remains attached to the tropomyosin filament. This invariant linkage strengthens the head-to-tail joint between molecules, enhancing the cooperativity of the thin filament and maintaining the conserved connection of troponin to tropomyosin required for the switching mechanism[8].

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Dependence of the mechanical properties of actin/α-actinin gels on deformation rate

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The cortical cytoplasm, including the cleavage furrow, is largely composed of a network of actin filaments that is rigid even as it is extensively deformed during cytokinesis[1,2]. Here we address the question of how actin-filament networks such as those in the cortex can be simultaneously rigid (solid-like) and fluid-like. Conventional explanations are that actin filaments rearrange by some combination of depolymerization and repolymerization; fragmentation and annealing of filaments; and inactivation and re-establishment of crosslinks between filaments[3-5]. We describe the mechanical properties of a model system consisting of actin filaments and *Acanthamoeba* α-actinin[6-8], one of several actin crosslinking proteins found in amoeba and other cells[4,9]. The results suggest another molecular mechanism that may account for the paradoxical mechanical properties of the cortex. When deformed rapidly, these mixtures are 40 times more rigid than actin filaments without α-actinin, but when deformed slowly these mixtures were indistinguishable from filaments alone. These time-dependent mechanical properties can be explained by multiple, rapidly rearranging α-actinin crosslinks between the actin filaments, a mechanism proposed by Frey-Wyssling[10] to account for the behaviour of cytoplasm long before the discovery of cytoplasmic actin or α-actinin. If other actin-filament crosslinking proteins behave like *Acanthamoeba* α-actinin, this mechanism may explain how the cortex recoils elastically from small rapid insults but deforms extensively when minute forces are applied over long periods of time[1,11,12]

We evaluated the mechanical properties of mixtures of actin and *Acanthamoeba* α-actinin in a cone-and-plate rheometer using a sinusoidal pattern of low amplitude deformations[8,13-15]. As described previously[15], the small strains (deformations) used do not alter the mechanical properties of equilibrated samples of actin.

It is known that α-actinin increases both the rigidity (elasticity, G') and viscosity (η') of actin filament[8], but we show here that the extent of these changes depends strongly on the rate of deformation (Fig. 1). At equilibrium, actin filaments alone at 24 μM form a weak gel (viscoelastic solid) characterized by a frequency-independent rigidity (equilibrium elastic modulus), $G_e = 14 \pm 4$ dyn cm^{-2} (Fig. 1*a*), as shown previously[15]. With the addition of α-actinin, both the dynamic elasticity G' and dynamic viscosity η' of rabbit actin filaments are elevated 40-fold when sinusoidally deformed (strained) at 1.0 Hz. But at low frequencies ($<10^{-3}$ Hz), both parameters approach values for actin filaments alone (Fig. 1*a*).

The material properties of mixtures of polymerized actin and α-actinin at high rates of sinusoidal deformation are attributable to multiple crosslinks between the filaments, as control experiments exclude substantial contributions by the other components in the mixture. First, free α-actinin, free actin filaments and free nonfilamentous actin do not contribute significantly to the mechanical properties of the gel because G' and η' for each alone are at least 10-fold lower at high frequencies ($>10^{-2}$ Hz) (Fig. 1). Second, complexes of α-actinin with actin monomers (if any) do not contribute, because α-actinin does not alter the rheological properties of nonfilamentous actin alone (Fig. 1*b*). Third, the temperature dependence of the elasticity of the mixture of α-actinin with actin filaments is opposite to that of the

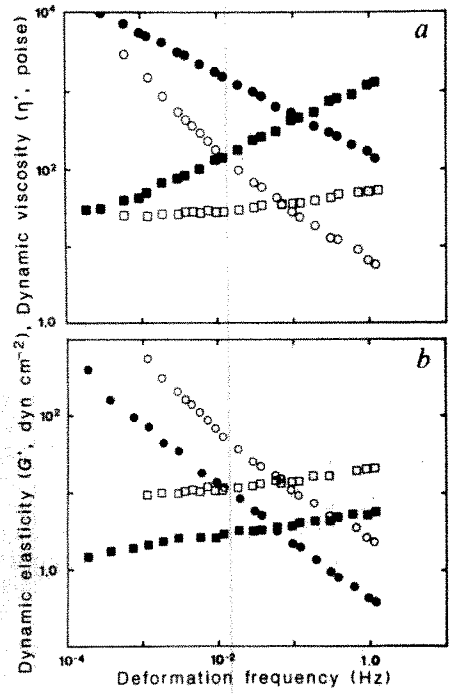

Fig. 1 Effect of *Acanthamoeba* α-actinin on the mechanical properties of actin over a wide range of deformation rates (frequencies). *a*, 24 µM filamentous actin alone (F-actin; white squares, G'; white circles, η') or filamentous actin plus 1.6 µM α-actinin (F-actin + α-actinin, black squares, G'; black circles, η'). *b*, 1.6 µM α-actinin in polymerization buffer (black squares, G'; black circles, η') and 24 µM nonfilamentous actin plus 1.6 µM α-actinin (white squares, G'; white circles, η') in low-salt buffer (0.1 mM $MgCl_2$, 1 mM EGTA, 2 mM PIPES *p*H 7.0, 0.2 mM ATP, 1 mM dithiothreitol (DTT), 0.5 mM NaN_3).

Methods. *a*, G' and η' were evaluated with a cone-and-plate rheometer (Weissenberg rheogoniometer) for the frequency range 10^{-4}–3 Hz. The sample was deformed by sinusoidally oscillating the bottom plate relative to a freely suspended cone using small displacements that do not disrupt the equilibrium mechanical properties of actin filaments[15]. From the phase and amplitude response of the cone relative to the plate, we calculated G' and η' using linear viscoelastic theory[15,19]. In this type of experiment, the freely suspended cone oscillates in phase with the plate if a solid is present between cone and plate. If a newtonian liquid like water is placed between cone and plate, the cone oscillates −90° out-of-phase with the plate. For a complex material like actin that exhibits both solid-like and liquid-like properties, dynamic elasticity and dynamic viscosity are the in-phase and −90° out-of-phase components of the cone response. These dynamic parameters measure elasticity and viscosity under nondisruptive conditions and have no relation to shear rate dependent parameters such as shear viscosity[15,19]. Gel-filtered actin from rabbit skeletal muscle[30] and *Acanthamoeba* α-actinin[6,7] were equilibrated in polymerization buffer (100 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 10 mM PIPES *p*H 7.0, 0.2 mM ATP, 1 mM DTT, 0.5 mM NaN_3) for 1,000 min at 25 °C in the rheometer[15]. The rheometer cone and plate were 10 cm in diameter with a cone angle of 0.01729 rad. The deformation frequency was varied with a constant amplitude of 0.0046 rad for a maximum strain of 0.083[15]. Below a frequency of 6×10^{-2} Hz, actin filaments have a frequency-independent elasticity G_e (see text), characteristic of a viscoelastic solid[15,19]. At 1.0 Hz, α-actinin increases both the G' and η' by 40-fold, but at 6×10^{-4} Hz the mixture was mechanically indistinguishable from actin filaments alone. These data were reproduced for two sets of experiments with two preparations of α-actinin. Both types of samples contained numerous actin filaments shown by electron microscopy[31]. *b*, G' tends toward zero at low frequency indicating that α-actinin alone is a viscoelastic liquid. G' and η' are identical for α-actinin in polymerization buffer and low-salt buffer. For the mixture of nonfilamentous actin and α-actinin, G' and η' are the same as values for nonfilamentous actin alone indicating that α-actinin does not significantly affect the mechanical properties of nonfilamentous actin for this frequency range.

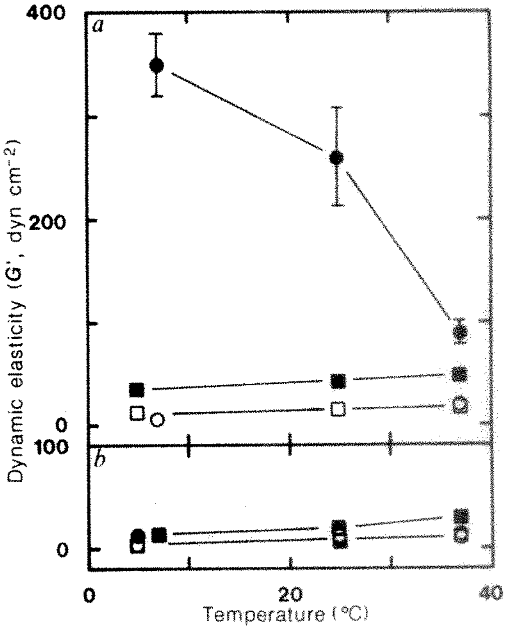

Fig. 2 Temperature effects on dynamic elasticity. 24 µM filamentous actin (G', black squares), 24 µM filamentous actin plus 1.6 µM α-actinin (black circles), 24 µM nonfilamentous actin (white squares), 24 µM nonfilamentous actin plus 1.6 µM α-actinin (white circles). These symbols correspond to the calculated means for 2–5 sets of experiments with proteins from different samples of *Acanthamoeba*. Standard deviations were smaller than the space occupied by the symbols, except where designated by error bars. *a*, Deformation frequency = 6×10^{-1} Hz. The mixture of filamentous actin plus α-actinin had higher values of G' and an opposite trend of G' as a function of temperature compared with the other samples, showing that functional crosslinks between actin filaments dominate the mechanical properties for the mixture at high frequencies. *b*, Deformation frequency = 6×10^{-4} Hz. For all samples, G' increased with temperature in a similar fashion.

other components (actin monomers ± α-actinin and actin filaments alone) in the sample (Fig. 2).

The key experiment to elucidate the frequency dependence of the mechanical properties of these gels was to measure the equilibrium binding constant for α-actinin to actin filaments (Fig. 3), so that we could estimate the steady-state association and dissociation rates of the crosslinks. In sedimentation binding experiments, the dissociation constant K_D of the actin filament and α-actinin complex is 26 µM (Fig. 3). From this value we estimated the number of α-actinin molecules bound and the possible exchange rates. First, under the conditions used in Figs 1 and 2, only 35% of the total α-actinin (1.6 µM) is bound to actin filaments; but as the average filament length is probably >5 µm (ref. 16) >50 α-actinins are bound to each filament at any instant. Second, if the association rate constant is assumed conservatively to be $10^5\ M^{-1}\ s^{-1}$, the dissociation rate constant is $>2\ s^{-1}$. The actual value may be one or two orders of magnitude higher, because other proteins that bind to actin filaments such as myosin have association rate constants of 10^6 to $10^7\ m^{-1}\ s^{-1}$. Consequently, at equilibrium the α-actinin in these gels binds to and dissociates from the actin filaments rapidly ($2–200\ s^{-1}$), like the weakly bound intermediates in the actomyosin crossbridge cycle in muscle contraction[17,18]. The difference with myosin, however, is that α-actinin 'cycling' is independent of ATP.

The rapid binding equilibrium of α-actinin with actin filaments means that crosslinks between filaments can rearrange quickly and together with the existence of multiple crosslinks between filaments explains why the mechanical properties of the gel depend on the rate of sinusoidal deformation (Fig. 1).

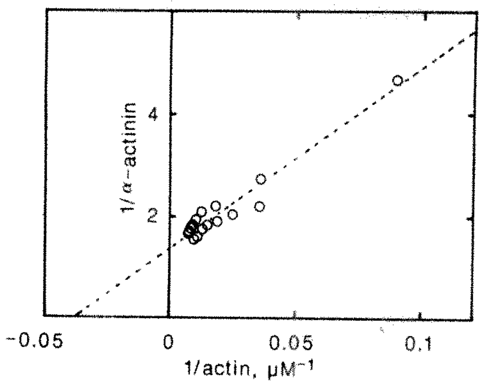

Fig. 3 Dissociation constant for the complex of *Acanthamoeba* α-actinin with actin filaments.

Methods. We incubated 8.5 nmol of α-actinin with 408 nmol [^{14}C]iodoacetamide (NEN, Boston) for 90 min in 10 mM Tris-HCl *p*H 7.5, 1 mM EDTA, 0.1 mM DTT in 1 ml total volume at room temperature. The reaction was stopped with 3 μmol DTT and the products were concentrated to 0.5 ml vol. by dialysing against Aquacide II (Calbiochem, La Jolla) for 60 min, 5 °C. The products were gel filtered on a S-300 column (1.2 × 47 cm) pre-equilibrated with Tris-EDTA-DTT buffer and radioactivity counted on a Beckman LS 7000 liquid scintillation counter. The labelling varied from 20 to 100% for three batches of α-actinin. The gelation activity was assayed with the 'falling ball' technique[32] and varied from 80 to 150% compared with unlabelled α-actinin. Iodoacetamide-labelled α-actinin (1.6 μM) was incubated for 1,000 min at 25 °C with various concentrations of rabbit muscle actin (5-180 μM) in polymerization buffer (100 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 10 mM PIPES *p*H 7.0, 0.2 mM ATP, 1 mM DTT, 0.5 mM NaN_3). The mixtures were centrifuged for 25 min at $1.6 \times 10^5 g$ in an Airfuge (Beckman) to pellet the actin filaments and the supernatants (70 μl) were assayed for radioactivity to measure the free α-actinin. The amount of α-actinin in the pellet was calculated from the original total and the data are presented as a double reciprocal plot by the method of Brenner *et al.*[17]. These data from two different batches of α-actinin were fit to a linear regression line. The intercept on the x axis gives $K_D = 26$ μM.

When force is applied slowly, the α-actinin crosslinks rearrange faster than the displacement of actin filaments relative to each other, so they offer little or no resistance. When force is applied more rapidly than the whole population of crosslinks can rearrange, there will be physical connections between the filaments that make the network rigid. Amazingly, Frey-Wyssling[10] in 1948 proposed a similar model without knowledge of cytoplasmic actin or crosslinking proteins.

Because association and dissociation rates are functions of temperature, the rate of crosslink re-arrangement should also increase with the temperature. This may explain why the rigidity (G') of the gel mixture decreases with temperature, first shown by Abe and Maruyama[8], whereas G' for the individual components increases with temperature (Fig. 2*a*). During low-frequency deformations this effect of temperature on the mixture is not observed because the rate of crosslink re-arrangement is faster than the deformation frequency (Fig. 2*b*). We have recently confirmed every aspect of this model for α-actinin in fluorescence photobleaching recovery experiments (M.S., D. Loftus, J. Cooper, E. Elson, C. Freider and T.P., in preparation).

The effects of α-actinin on the mechanical properties of actin filaments were unexpected because, to our knowledge, similar effects have not been observed for either synthetic polymers or isolated proteins. Addition of covalent crosslinkers to a viscoelastic solid of synthetic polymers results in the elevation of the equilibrium elastic modulus (G_e) rather than only an increase in G' at high frequency[19]. Although actin-filament crosslinking proteins such as α-actinin, actin-binding protein, spectrin and others[4,9] do not covalently link filaments, previous experimental and theoretical studies of filament networks have assumed for simplicity that the crosslinks are permanent[20-23]. In dynamic models of cytoskeletal gels, for example, crosslinking proteins have been modelled as permanent links that are disrupted by regulatory factors such as calcium[22,23]. Our data show that the network of *Acanthamoeba* α-actinin and actin filaments is both rigid and deformable depending on the rate of sinusoidal deformation without requiring regulation by inactivating crosslin fragmenting filaments or depolymerizing filaments. This may b particularly important for *Acanthamoeba* α-actinin, as it is not regulated by calcium[7].

Macrophage α-actinin[24] and actin-binding protein[25] are also likely to be in rapid equilibrium with actin filaments because they bind relatively weakly ($K_D = 0.2$–0.5 μM), especially α-actinin in the presence of calcium ($K_D = 1$ μM). As discussed above, the dissociation rate constants may be in the range of 0.02–10 s^{-1}. Consequently, the mechanical properties of actin filaments with these crosslinkers should also depend on the rate of deformation. This notion of dynamic crosslinks is consistent with the recent data of Zaner[14,26] and should become more obvious at lower and lower frequencies.

The dynamic model[10] for α-actinin crosslinking of actin filaments may be applicable to the amoeba cortex and may explain some features of cytokinesis. Like amoebas[7,27] both sea urchin eggs[28] and cultured vertebrate cells[29] have a cortical cytoplasm that is rich in actin filaments and α-actinin. Hiramoto[1] and Peterson *et al.*[12] showed that the cortex of sea urchin eggs and fibroblasts is rigid (elastic) and more resistant to rapid deformations than to slow deformations. For example, although the cleavage furrow generates only minute forces of 3×10^{-3} dyn or stresses between 10 and 50 dyn cm^{-2} over 10–30 min (between 10^{-3} and 10^{-4} Hz), it is capable of grossly deforming the highly rigid cortex[1]. Our data suggest that α-actinin localized to the cortex[27-29] forms an actin gel that resists rapid insults yet deforms with minimal resistance provided that stress is applied for a long time.

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Mapping using gene encyclopaedias

D.L. Daniels and F.R. Blattner

Overlap cloning and large fragment electrophoresis bring total knowledge of the E. coli *genome into prospect.*

THE circular genome of *Escherichia coli* consists of 4.7 million basepairs ± 100 kilobase pairs, corresponding to a genetic map of 100 minutes. Over 1,000 genes are mapped[1], and perhaps 3,000 remain to be discovered. Recently, a restriction map has been determined using *Not*I and *Sfi*I, having 23 and 27 cleavage sites, respectively (C.L. Smith and C.R. Cantor, Columbia University, personal communication.) This map provides the most accurate measurement of the genome size.

During the past five years our laboratory has been working on a programme to create an ordered set of DNA clones spanning the genome of *E. coli*. The term "gene encyclopaedia" is suggested for this organized clone bank to distinguish it from a "library", which is a random collection. Similar efforts have been initiated by Olson *et al.*[2] for yeast, and by Coulson *et al.*[3] for the nematode. George Brownlee at Oxford has also recently started an *E. coli* cosmid project along similar lines (personal communication).

Utility

The *E. coli* gene encyclopaedia, when completed, will provide researchers with important advantages. For example, if the map position is known for a desired gene it could be "cloned by phone". If not, it could still be isolated more quickly by screening an encyclopaedia than a library, as fewer individual clones would need to be screened to ensure coverage of the entire genome. Moreover, a mutation with a phenotype that cannot be detected in clones could be genetically mapped using the encyclopaedia clones in marker rescue tests, thereby identifying a clone of the gene.

The encyclopaedia could also be used to study gene regulation in *E. coli*. For example, to identify all the genes that are under the control of a particular regulatory mechanism such as catabolite repression, the SOS pathway or heat shock, the encyclopaedia could be screened with RNA probes synthesized in *E. coli* in response to the inducing stimulus. Once identified, the clones could be used for quantitative studies. Regions lacking regulated genes could also be demonstrated. In the past, characterization of pleiotropic regulatory systems has required individual testing of postulated targets.

Finally, the encyclopaedia will aid in physical mapping of the genome, including its sequencing, and facilitate engineering modification. Approximately eight per cent of *E. coli* has been sequenced so far through uncoordinated individual efforts.

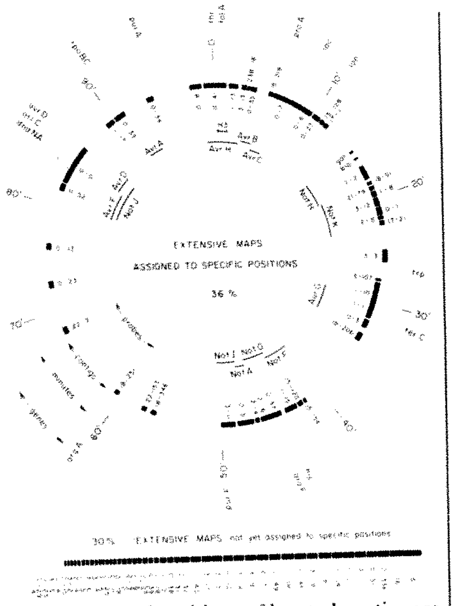
EXTENSIVE MAPS
ASSIGNED TO SPECIFIC POSITIONS
36 %
30 % EXTENSIVE MAPS not yet assigned to specific positions

Fig. 1 Sizes and positions of located contigs are indicated on the circular map. Extensive maps which have not yet been located are shown below the circle.

Overlap cloning

E. coli K-12 strain MG1655 was chosen as the reference strain after consultation with Barbara Bachman of the Yale University Microbial Stock Center, because it is prototrophic, has not been artificially mutagenized, and lacks gamma delta sequences, which are known to promote DNA rearrangement.[4]

A minimal encyclopaedia would require about 125 cosmid clones or 250 phage lambda clones. Lambda vectors were chosen despite their smaller capacity, out of concern for accuracy and completeness. In general, cosmid and plasmid clones may accumulate deletions as their rate of doubling depends inversely on DNA length, and because expression of cloned genes may be selected against due to deleterious metabolic imbalances. This could be especially true in the more useful high copy number cosmids.

Phage lambda, on the other hand, destroys its host in ten minutes, and thus need not coexist with it. Moreover, there is inherent selection against deletion in lambda clones due to the existence of a lower limit on the size of DNA that can be propagated. Charon 28[5] and Charon 40[6] were used. The latter vector has a 24 kilobase pair DNA capacity and offers a very convenient polystuffer method for isolation of vector arms[6].

The initial approach resembled the 'random' method used for DNA sequencing. Three types of libraries were made from partial digests of *E. coli* DNA with *Bam*HI, *Eco*RI, and *Hin*dIII. CsCl gradient centrifugation of the packaged phage enriched the libraries for large DNA inserts. Individual clones were chosen at random, cut with each of the three enzymes and pairs of enzymes, and mapping gels were run. Fragment sizes were measured using a digitizer and marker calibration program. The results were fed into a computer which was programmed to search for overlaps between clones.

The 'signature' used to identify overlaps consisted of a list of fragment sizes for each clone plus the binary data of whether the fragment was cut by each of the other enzymes. The program scanned the database for pairs of clones sharing at least two signature elements and overlapping maps were assembled from amongst the possibilities by hand.

Selection of the signature was tricky. A prime consideration was the desire to obtain a complete *Bam*HI–*Eco*RI–*Hin*dIII map of *E. coli* as a byproduct. The order and position of restriction sites in most of the clones was determined, but some of the maps were indeterminate. Thus the signature used by the program was an unordered one that could be obtained from the data regardless of the state of the mapping. The disadvantage of this was that signature elements in the middle of the cloned insert were not distinguishable from those at the ends, so the strongest matches corresponded to the less interesting duplicate clonings, whereas the interesting partial overlaps had to be sorted out from the noise of chance fragment identities.

Although a 'denser' unordered signature such as that of Coulson *et al.*[3] might be beneficial, in retrospect an ordered signature would have been much more powerful. A set of partial restriction enzyme digests run by the Smith and Birnstiel[7] method in a field inversion gel could be a good choice for future efforts.

Data for 2,000 clones has been coalesced into 68 overlapping contiguous regions or 'contigs' ranging in size from 18 to 200 kilobase pairs, accounting for 67 per cent of the genome. Much of the remainder is probably covered by the over 250 unique unattached clones that remain in the collection.

Field inversion gels

The advent of orthogonal field[8] and field inversion[9] gel electrophoresis for separating large DNA molecules allowed a quantum leap in assembly of the encyclopaedia. Using field inversion gels DNA fragments of 100 to 500 kilobase pairs were isolated from restriction digests with *Not*I, *Sfi*I, *Spe*I, *Avr*II and *Hin*dIII. These were used as hybridization probes to spot blots of the entire clone bank. The resulting data helped order the collection by identifying neighboring contigs. In many cases this allowed the discovery of new overlaps. For example, overlaps of single fragments could often be identified in the context of a few hundred kilobase pair subregions. This approach also allowed the sizes of remaining gaps to be estimated by subtraction. The positions of the gaps could often be pinpointed as well. In general, gaps were small, and we suspect in many cases that contigs abut without an overlapping clone to prove it.

Genetic map assignment

The literature was surveyed for restriction maps of known genes, and a computer program located the corresponding clones. Other clones were located by genetic tests such as the elucidation of those carrying *lacZ* by their formation of blue plaques, and the ability of lambda clones carrying the wild type allele to plate on *groE, dnaK and dnaJ* bacteria. Tests for other markers prove to be laborious, however, and have not been used extensively.

Figure 1 shows the state of the encyclopaedia as of 30 January 1987. The genetically assigned clones are positioned around the circle, and correspond to 36 per cent of its length. The ones across the bottom depict unassigned extensive contigs, corresponding to 30 per cent of the genome. It is to be expected that their assignment by hybridization will be accomplished soon.

After all the isolated clones have been assigned, gaps will remain, possibly due to awkward restriction site placement. It will be fascinating to examine the gaps that remain after the trivial gaps have been filled, for these nonclonable regions may carry biologically interesting structures.

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ASM anecdotes

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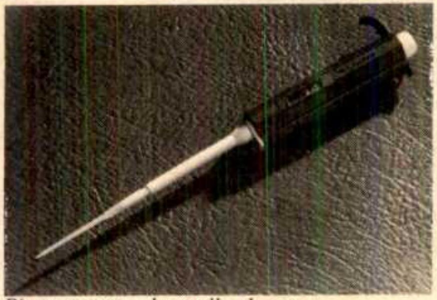

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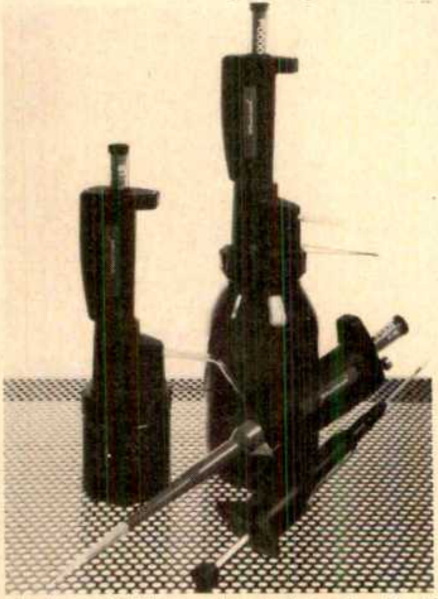

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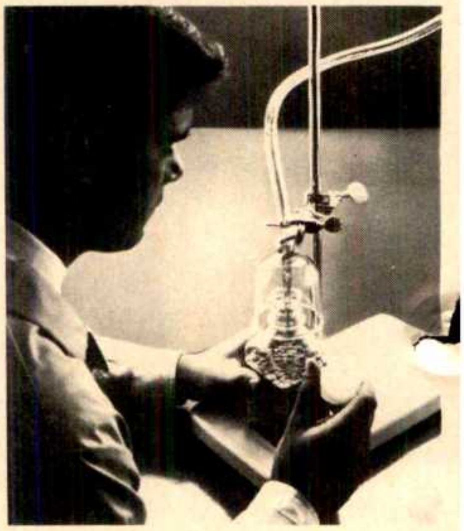

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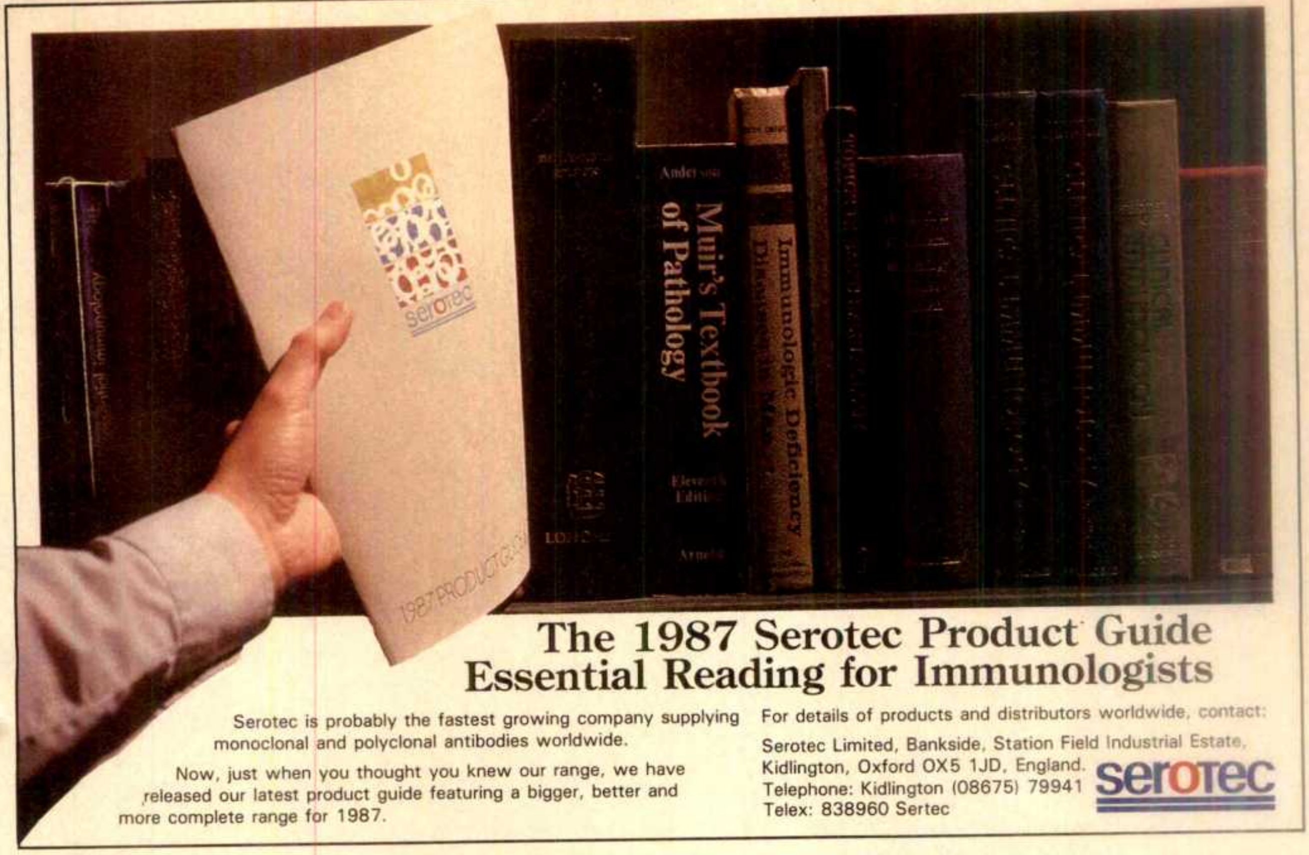

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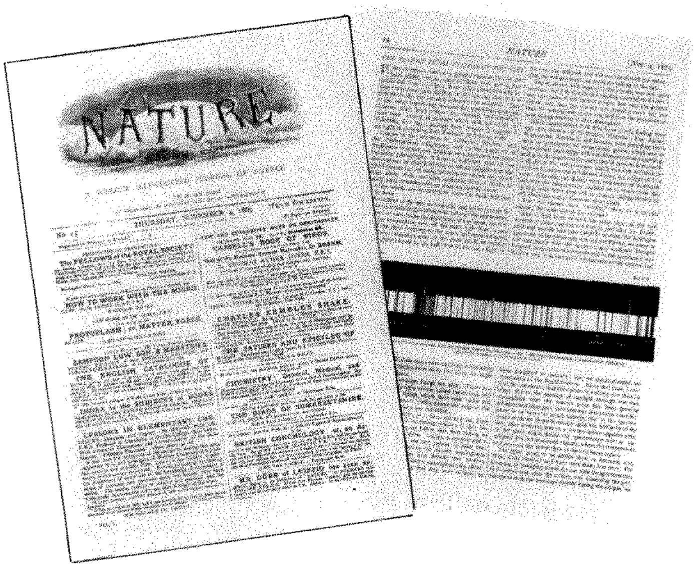

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media. PacketMedia pouches come in sizes for the production of 500 ml, 1 litre and 2 litres of prepared media, and most have a half-life of four to five years. Information on the range of University Micro Reference Laboratory's media can be found in its booth, number 1752.

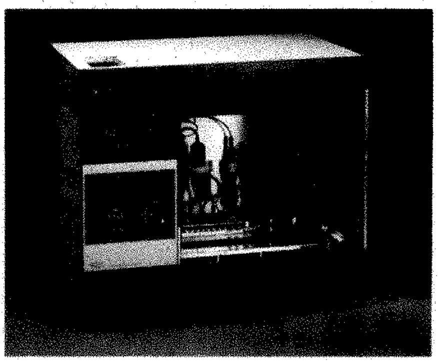
Latex agglutination tests are available from Wellcome Diagnostics, who will be exhibiting in island 7D (Reader Service No. 105). The tests work by using latex particles coated with group-specific antibody, and show results (visible agglutination) when the corresponding bacterial antigen reacts with the particles. Wellcome's tests, ranging in price from $77–205 (US), allow detection of A, B, C, D, F, and G *Streptococci*, *Staphylococcus aureus*, *H. influenzae*, *S. pneumoniae*, and *N. meningitidis*.

Detects bacteria by visible agglutination.

Flow Laboratories has introduced a new range of cell growth products manufactured by Collaborative Research, Inc. (Reader Service No. 106). Included in the range are broad purpose mitogens, biomatrix products, formulated medium specialities, and serum-free media additives. These products are provided in sterile, lyophilized pack sizes for propagation and differentiation of human and animal cells in culture. Flow Laboratories' cell culture products will be in island 7C and booth numbers 849 and 850.

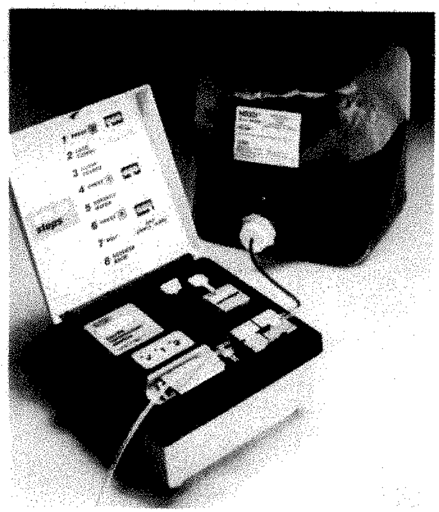
Fermenting focus

Charles River Biotechnological Services will have information on its 5200R Opticell Culture System in booth numbers 1505 and 1506. (Reader Service No. 107). The $28,750 (US) system is a benchtop version of the larger Opticell 5300 and 5400, and is designed to produce monoclonal antibodies and other biologicals in research quantities, to serve as a process development tool. The 5200R exhibits real-time feedback, and is suitable for the culture of attachment-dependent or suspension cells. The Opticell 5200R has several features not found on the larger systems. The system is available with a dual loop option, allowing two complete bioprocess shelves to share the same process controller, for a truly controlled experiment without the added cost of having two separate culture systems. The operator also has independent control of the parameter settings for each loop, such as dissolved O_2, *p*H, temperature and feed/harvest rates. The system is self-contained, with a clean air curtain to avoid contamination.



A controlled culture system that fits right on the benchtop.

Designed to make rapid sterile connections in cell culture or fermentation systems, the Du Pont SCD-IIB sterile connection device is also compact and portable (Reader Service No. 108). Using the $9,450 (US) SCD-IIB, it is possible to add liquids or media, sample fermenter and receiver contents, replace fermenter components, harvest products, or transfer cells between containers, in a few steps. Du Pont's sterile connection device operates by providing sterile butt welds between ends of thermoplastic tubing, protecting the integrity of bioreactor systems, and Du Pont claims its device has never failed in a test of 3,000 connections of 300 individual cultures. Du Pont will be exhibiting its wares in island 13C.



Du Pont's SCD-IIB really makes the connection.

Fisher Scientific's new line of laboratory circulators all share a common feature, according to Fisher: a high performance to price ratio (Reader Service No. 109). The line includes six immersion, three heating, and six refrigerated units,

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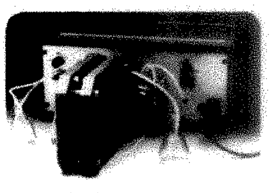

Our new LIPOSOMAT guarantees rapid (5ml within 60 minutes) preparation of uniformly sized unilamellar liposomes. Liposome size is selectable between 25 and ca. 600 nm diameter. Sample volume is normally 5-10ml or up to 200ml if used in combination with an additional instrument. Lipid concentration can be up to 300 mg/ml.

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Reader Service No.47

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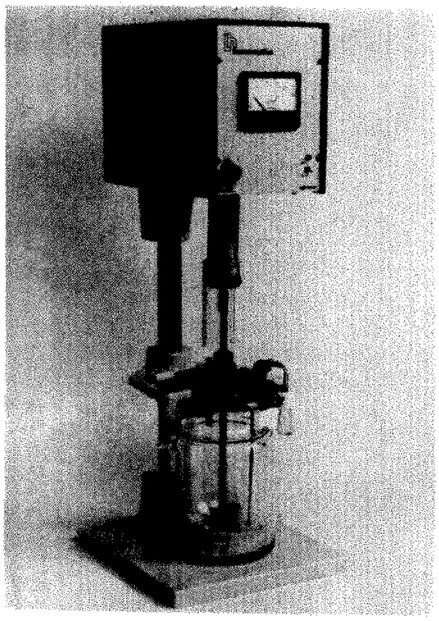
available for $350–$1875 (US), $760–$1135 (US), and $1325–$2395 (US), respectively. The units offer temperature control to ±0.02 °C via proportional heating, user-adjustable pre-set temperatures, stainless-steel components, resettable circuit breakers, 1,000 W heaters, and selectable pump speeds of between 7 and 15 litres per minute. Fisher Scientific will have its range of products for the microbiology laboratory in booth numbers 1010, 1011 and 1052–1055.

For the cultivation of shear-sensitive cells, LH Fermentation, in booth numbers 1856 and 1857, sells a direct-drive, slow-speed stirrer (Reader Service No. 110). The Model 502D features marine impellers with variable speeds between 0 and 250 r.p.m., and a dome-based culture vessel — all designed to treat sensitive cultures gently. The 1.3 to 2.3 litre vessels are provided with a flat-bottomed jacket that allows them to stand flat on the benchtop, that can be connected to a thermocirculator for temperature control. All the normal growth parameters can be monitored with LH Fermentation's bioreactor, that can be coupled to the 500 Series modular control systems for further automation.


Features a marine impeller to be easy on cells.

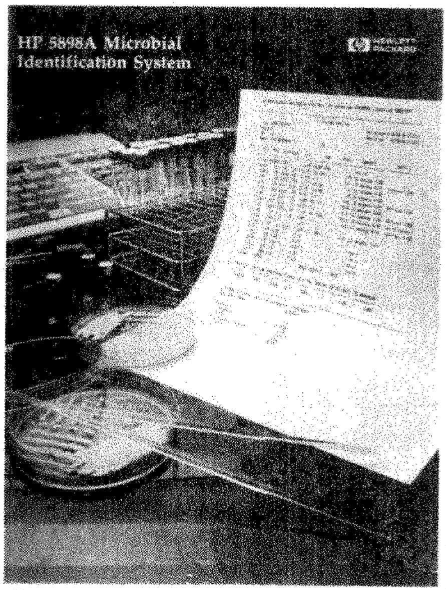
Culture conveniences

Promega announces the addition of a new six-extract package size to its Packagene **lambda DNA packaging system** (Reader Service No. 111). According to Promega, the Packagene system is a high efficiency, single-extract *in vitro* lambda packaging system that is easy to use because it eliminates unnecessary steps and packages up to 5 μg of DNA. The six-extract size $145 (US) Packagene system also includes control lambda DNA and the LE392 plating strain.

DNASTAR Inc. has entered into a joint venture with Field Inversion Technologies Corp. to market products based upon the **field inversion gel electrophoresis** technology developed by G.F. Carle, M. Frank and M.V. Olson at Washington University (Reader Service No. 112). DNASTAR Pulse is a computer-driven controller that provides field inversion capability for up to four independent experiments simultaneously, and separates DNA molecules of up to two million base pairs. The electrophoresis programmer allows researchers to set electric fields in forward and reverse directions and to program the linear ramps optimal for the size range of given molecules. The DNASTAR Pulse system's $1,490 (US) price includes the program, the program controller, the driver interface card, and the electrophoresis programmer with one switch module.

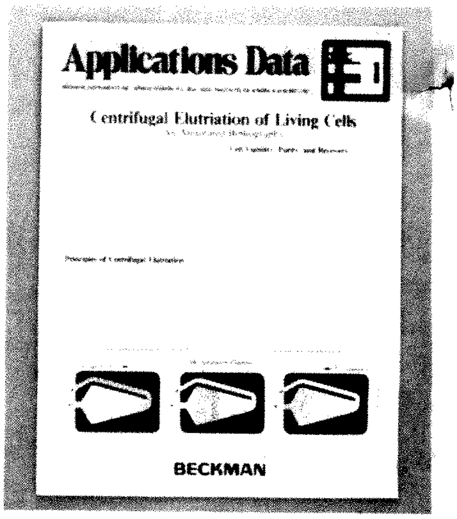
Throughout the year, the American Type Culture Collection, in booth number 1504, will be holding a series of workshops at its facilities in Rockville, Maryland (Reader Service No. 113). From February to June, the topics for these workshops will include freezing and freeze-drying of microorganisms, recombinant DNA, *Acanthamoeba* and *Naegleria* infections, gas chromatographic analysis of cellular fatty acids for bacterial identification, freezing and quality control of cell cultures and hybridomas, clinical anaerobic bacteriology, and biotechnology patents.

Hewlett-Packard has expanded its HP 5898A Automated Microbial Identification System to include an anaerobic database library containing more than 50 groups of anaerobic bacteria (Reader Service No. 114). This provides an automatic method for the computerized identification of microbes by means of their characteristic fatty acid profile under gas chromatography, and together with the existing database of 178 aerobic species and subspecies, makes for a complete identification system. Anaerobes included in the new database include actinomyces, bacteroides, clostridium, fusobacterium, propionibacterium and veillonella. The library is available on floppy disk as a $3,000 (US) option, and will be updated regularly. New libraries will be added as they become available.



The booklet on HP's Microbial Identification System.

To aid in the recovery and harvesting of whole living cells, Beckman has come out with a new bibliographic guide on centrifugal elutriation (Reader Service No. 115). The 24-page booklet includes more than 700 references documenting the use of Beckman elutriation systems for harvesting cells. The free booklet is divided into 27 major categories, including yeast cells, drug suspensions and subcellular fractions. All procedures referenced in the booklet use Beckman Model JE-6B and JF-10X Elutriator Rotors. Beckman will have a full line up of its products for centrifugation in island 4E and booth numbers 422–426.


Applications Data

Centrifugal Elutriation of Living Cells

BECKMAN

Tips on spinning and harvesting cells.

A desk-pad sized **metabolic chart** is available free of charge from Koch-Light (Reader Service No. 116). The 35 × 29 cm chart, published with the cooperation of Donald Nicholson, outlines pathways of biosynthesis and degradation of over 500 compounds. To ensure that the metabolism of carbohydrates, lipids, amino acids, and purines and pyrimidines are differentiated, and their interrelationships are made clear, the chart is printed in full colour. Koch-Light also publishes a range of charts and booklets as teaching aids, and these products are described in the current Koch-Light catalogue.

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated.

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This is a non-renewable academic position available for up to five years. We require a cell biologist to join a group investigating an in vitro transdifferentiation and regeneration system based entirely on striated muscle cells of jelly fish.

The candidate should have a PhD and a good background in biochemistry. His/her work will focus on the mechanisms which stabilise cells in the differentiated state. It will include biochemical analysis of extracellular matrix components and application of mAb and standard cell biological techniques to early transdifferentiating cells. Some teaching duties are included. Salary is according to experience.

Applications including a cv and the names of two referees should be sent to: **Erziehungsdepartement Basel-Stadt, Personalabteilung I, Münsterplatz 2, CH 4051 Basel.**

(W3834)A

SCIENTIFIC PROGRAMMER III

Knowledge Systems Laboratory is seeking applicants for the position of Scientific Programmer with the Heuristic Programming Project. This Programmer will be part of a team of researchers investigating concurrent systems for expert systems. Primary responsibilities include: 1) transfer of knowledge to research team regarding current Japanese efforts in the Fifth Generation Project, 2) development and maintenance of an architectural simulation system, 3) aid in the development of tools to support concurrent problem-solving frameworks that will run on the simulator, and 4) maintenance of the HPP Japanese Library which includes occasional translation of technical material.

Specific qualifications include experience in Lisp programming on the Symbolics 3600 and TI Explorer with a background in artificial intelligence applications, as well as in object-oriented graphics programming and experience in programming for and programming simulations of multi-processor systems. Candidate must be bilingual in English and Japanese. MS degree in Computer Science. Experience, however, is not required. Job site and interview Stanford, Calif. 40/hrs. per wk. Salary $38,690/yr. Send ad and resume to MLU #12001 PO Box 9560, Sacramento, Calif. 95823-0560 not later than March 13, 1987.

(NW533)A

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(2236)A

POSTDOCTORAL RESEARCH POSITIONS

LA JOLLA CANCER RESEARCH FOUNDATION

The La Jolla Cancer Research Foundation is a growing research institute located in the Torrey Pines Research Park, in close proximity to the Salk Institute, Scripps Clinic and Research Foundation, and the University of California, San Diego. The Foundation has been designated as a Cancer Research Center by the National Cancer Institute. The research of the Foundation's staff utilizes molecular, biochemical, and cellular approaches and focuses on the relationships of cancer and mammalian development. Postdoctoral research positions are supported by individual investigators and an NCI postdoctoral training program in Developmental Gene Regulation.

Senior graduate students are encouraged to apply for these positions. For more information, inquiries should be addressed to either individual investigators or the Director, Postdoctoral Training Program.

Staff Members

Eileen Adamson, Ph.D.,
Staff Scientist
Expression of oncogenes and growth factor receptors during development; differentiation of teratocarcinoma cells.

Janice Buss, Ph.D.,
Assistant Staff Scientist
Role of fatty acid acylation of proteins in cellular transformation and protein:membrane interactions.

Channing Der, Ph.D.,
Assistant Staff Scientist
Role of cellular oncogenes in neoplastic transformation and cellular differentiation.

Eva Engvall, Ph.D., Staff Scientist
Composition, structure and function of basement membranes and extracellular matrices.

William Fishman, Ph.D.,
President, Staff Scientist
Tumor marker studies: Placental genes operating in cancer cells.

Frank Fujimura, Ph.D.,
Assistant Staff Scientist
Viruses and teratocarcinoma differentiation.

Michiko N. Fukuda, Ph.D.,
Staff Scientist
Role of membrane glycoproteins in embryonic development.

Minoru Fukuda, Ph.D.,
Staff Scientist
Cell and molecular biology of glycoproteins in differentiation and oncogenesis.

Paul Goetinck, Ph.D.,
Staff Scientist
Extracellular matrices in development; regulation of expression and structure-function relationships.

Terumi Kohwi-Shigematsu, Ph.D.,
Assistant Staff Scientist
Chromatin structure and mechanisms of chemical carcinogenesis.

Waldemar Lernhardt, Ph.D.,
Assistant Staff Scientist
Molecular mechanisms regulating B lymphocyte proliferation.

Richard Maki, Ph.D.,
Staff Scientist
Genes involved in the immune response.

Elizabeth Mather, Ph.D.,
Assistant Staff Scientist
Molecular mechanisms regulating immunoglobulin gene expression.

José Luis Millán, Ph.D.,
Assistant Staff Scientist
Tissue-specific expression of genes in testis and placenta and their reactivation in cancer cells.

Robert G. Oshima, Ph.D.,
Staff Scientist; Director,
Postdoctoral Training Program
Early developmental regulation of cytoskeletal proteins; retroviral expression of antisense keratins.

Magnus Pfahl, Ph.D.,
Staff Scientist
*Characterization of new members of the steroid hormone receptor–*erbA *gene family.*

Michael Pierschbacher, Ph.D.,
Staff Scientist
Effects of cell adhesion on differentiative events.

Barbel Ranscht, Ph.D.,
Assistant Staff Scientist
Transmembrane linkage between the cytoskeleton and the extracellular environment of developing neurons.

William Raschke, Ph.D.,
Staff Scientist
Molecular analysis of lymphocyte differentiation.

Erkki Ruoslahti, M.D.,
Scientific Director
Structure and function of cell adhesion molecules and their receptors.

William Stallcup, Ph.D.,
Staff Scientist
Cell–cell interactions in the developing nervous system.

Charles Van Beveren, Ph.D.,
Assistant Staff Scientist
Oncogenic transformation by growth factor receptors.

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Stratagene is an innovator in research products for molecular biology. The company is now expanding its focus and plans to utilize its proprietary technologies in areas related to gene prognosis and gene expression in transgenic animals. Positions are available at several levels.

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Research Associate Applicants should have a B.S. or M.S. in Molecular Biology or Biochemistry and 2 to 3 years of relevant laboratory experience. Experience with **DNA sequencing**, nucleic acid hybridization, gene cloning and subcloning, is expected. Candidates should have the ability to function independently within the laboratory setting. Good interpersonal skills are required.

Stratagene provides a unique working environment. The company is growing rapidly and encourages group involvement in major decisions and policy. A generous stock option plan is available, in addition to standard fringe benefits. Stratagene is located within an important center for biotechnology. It is three miles from the University of California and several other major research institutes. Recreational opportunities are plentiful, being just minutes from the beach. Salaries are excellent.

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Please send resume and three references to Ms. Candra Carter, Stratagene Cloning Systems, 3770 Tansy Street, San Diego, CA 92121. (NW532)A

IMREG, INC.

DIVISION DIRECTOR MOLECULAR BIOLOGY

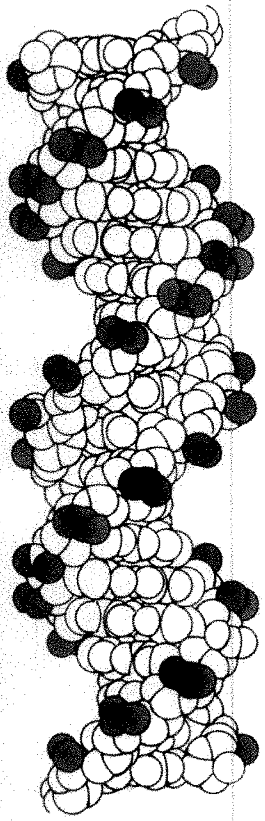
Imreg, Inc., a publicly traded biotechnology company, is a leader in the development of immunoregulators and diagnostics for human and veterinary applications. To support our continued growth, we are looking for a Director of our Division of Molecular Biology with excellent training, postdoctoral experience, and accomplishments in Molecular Biology, including recombinant DNA technology, gene cloning, and DNA sequencing.

Imreg, Inc. is developing unique technologies for clinically important problems which demand imaginative research and development, creating better medicines through immunology. We are looking for individuals who desire intellectually challenging positions to work at the advancing edge of recombinant DNA technologies as applied to problems in immunoregulation. The position is available at our Cambridge, MA facility. Candidates should be able to work independently, and have had experience in organizing and supervising a laboratory group.

Imreg, Inc. offers competitive salaries commensurate with training, experience, and record of research productivity, as well as an attractive fringe benefit package including a stock option plan, and linkages to the national academic community.

Please send curriculum vitae in confidence to John L. Carlton, Vice President of Administration and Finance, Imreg. Inc., Suite 1400, 144 Elk Place, New Orleans, LA 70112. (NW545)A

Head of the Department of Genetics



Glaxo Group Research Limited is Britain's leading pharmaceutical research organisation, with an international reputation for the discovery and development of safe and effective medicines.

In order to capitalise on recent research discoveries, we are expanding our genetics unit and creating a vacancy for the position of Head of a new Department of Genetics based at Greenford, which will comprise about 40 scientists and technicians. It will be part of the Molecular Sciences Division of Mammalian Biology and will have responsibility for the development of genetics research at the molecular, cellular, and whole organism level in bacterial, fungal, viral and animal systems.

Reporting to the Director of Mammalian Biology, this challenging new opportunity calls for an individual of significant professional stature, with proven abilities in one or more of these areas and who has an understanding of the broad range of genetics and its potential application in drug research. Furthermore, the successful candidate will be expected to enact the proposed expansion, and therefore should have proven experience of managing a substantial group of staff. Consequently we are looking for a dynamic and imaginative scientist with strong leadership abilities, who will join a management team comprising biologists and chemists, and who will be expected to contribute fully to strategic and tactical planning of research projects. The likely age range will be between 30 and 35 years.

As this is regarded as a key appointment, the benefits package will reflect its importance and include a Company car, free medical insurance, non-contributory pension and life insurance schemes. Full removal expenses will be re-imbursed if a move of home is necessary.

Candidates should forward a comprehensive curriculum vitae and publications list, or telephone for an application form to: Dr Martin Williams, Site Personnel Manager, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE. Telephone 01-422 3434 extension 2571 quoting reference number 010. (2235)A

Glaxo Group Research Ltd.

expert knowledge of physiology and pharmacology

Applications are invited for a research scientist position with a leading French DRUG COMPANY that is located in PARIS.

Candidates should have an expert knowledge of physiology and PHARMACOLOGY of the peripheral and central mechanisms of pain and analgesics.

Candidates should hold a Ph. D. or an equivalent degree and have substantial experience in research on pain and analgesics.

Applicants submit their C.V. with statement of major research interests under ref. 4578/NA to

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ROYAL MILITARY COLLEGE OF SCIENCE

Shrivenham

CHEMIST/BIOCHEMIST RESEARCH IN RADIOPROTECTION

Applications are invited for a post-doctoral research position, within the Chemical Systems Group of the School of Mechanical, Materials and Civil Engineering, in the area of chemical modifiers to confer radiation protection to cells. Experience in this field would be an obvious advantage and the programme of work could be modified to suit the individuals particular expertise. However, those interested in the chemical aspects of biochemistry and have a good practical ability are most welcome to apply.

The appointment will be on the RO1A Scale according to qualifications and experience (£8,020 — £12,780 per annum (under review)). The initial period of employment will be for one year which may be extended for up to three years.

RMCS is situated between Swindon (7 miles) and Oxford (20 miles) in the Oxfordshire countryside. There are good road and rail links.

Application forms may be obtained from the Personnel Office (HQ), RMCS, Shrivenham, Swindon, Wilts, SN6 8LA (0793) 782551 Ext 2421/2403 quoting reference SMMCE 26/86. Closing date: 19th March 1987. (2237)A

Research Scientists

Graduate Molecular Biologists

The Corporate Bioscience Group carries out research and development in the latest molecular and cellular biological techniques in support of ICI's biological business interests, principally in the health care area.

Our cancer molecular biology programmes now require two research scientists to join established teams (i) in the cloning, expression and modification of plant toxin genes for the production of improved immunotoxins and (ii) in investigating changes in gene expression in human breast cancer. Applicants should have graduate or equivalent qualifications in molecular biology, biochemistry, genetics or microbiology. Preference will be given to applicants with experience in recombinant DNA technology. We are seeking confident, self-motivated individuals with good communications skills and a genuine feel for this exciting area of scientific development.

Initially located at Runcorn Heath, Cheshire you will subsequently transfer when the group moves to new laboratory facilities at Alderley Park, Cheshire in 1988.

Salary will be in the range £8,800—£10,800 per annum, depending on qualifications and experience. Other benefits include a company profit sharing scheme, contributory pension fund and assistance with relocation where appropriate.

Please apply in writing, enclosing a full curriculum vitae and quoting reference RSCB to:
R E Neale, Personnel Officer, Corporate Bioscience Group, ICI plc, P O Box 11,
The Heath, RUNCORN, Cheshire
WA7 4QE. Closing date for applications: 25 March 1987.

ICI (2230)A

Corporate Bioscience Group

UNIVERSITY OF LEIDEN

The Center for Bio-Pharmaceutical Sciences has a vacancy for a

post-doc (m/f)

vacancy number: 7-005/2407

for the physicochemical characterization of drug delivery systems based on hydrophilic polymers, using relaxation N.M.R.

The work will mainly consist of:

a. studying the structure, on a molecular level, of the water-polymer system in relation to its composition;
b. studying the diffusional behaviour of drugs and model compounds in the system.

The applicant will perform the research in close cooperation with the Department of Physical Chemistry of the University of Leiden.

Required:
Ph.D. or ample experience in the field of relaxation N.M.R.

Information:
Prof. dr. H. E. Junginger, phone: 071-274308;
Prof. dr. J. C. Leyte, phone: 071-274243.

Candidates are invited to send a written application, with full C.V., to Directeur-Beheerder Gorlaeus Laboratoria,
Einsteinweg 5, Postbus 9502,
2300 RA LEIDEN. The Netherlands.

(W3845)A

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Enzymologists

in Canada

Located within easy access of metropolitan Toronto, Syntex Research (Canada) is devoted to the development of avant-garde strategies for the inhibition of clinically relevant enzymes. Since the inauguration of our facility five years ago, we have recruited a group of dynamic scientists who are dedicated to advancing the fields of medicinal chemistry and enzymology, and who place a high premium on novelty and innovation.

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Ph.D. Enzymologists

We invite applications from creative scientists interested in the design and assay of mechanism-based inhibitors, and who possess intensive training in enzymology. Our ideal candidate will have a good understanding of organic chemistry as well as expertise in enzyme kinetics, protein purification and modification, and be computer literate.

Our salaries and benefits package demonstrates our desire to attract the very best people possible.

For confidential consideration, please forward your resume to:
Jo-Ellen Robertson, Human Resources Specialist, Syntex Inc., 2100 Syntex Court, Mississauga, Ontario, Canada L5N 3X4

We are an equal opportunity employer

(NW556)A

BIOSEM

MOLECULAR AND CELLULAR BIOLOGY LABORATORY

Our biotechnology laboratory located in the University campus of Clermont-Ferrand is involved in the use of modern biology for improvement of plant varieties.
In order to complete our team of 14 scientists and technicians, we are lookind for :

A senior research scientist (ph D)

A research scientist (MSc)

Applicants must have a strong research experience in the field of plant cell culture technic and somatic embryogenesis.
A reasonable fluency of French should be an advantage for the applicants. These positions are offered in Clermont-Ferrand a city of the French Massif Central with a very interesting scientifical and natural environment.
Interested and qualified candidates are invited to submit curriculum vitae and references to :
GROUPE LIMAGRAIN - Département du Personnel - BP 1 - 63720 ENNEZAT (FRANCE).

(W3839)A

MEDIA PA

Groupe Limagrain

Position for

Research Assistant

with expertise in **immunology/molecular biology**.

Salary in the range of **£12.000—£21.000**

for Paris Laboratory

The successful candidate should be a non-smoking, ties-free, ambitious, young independent scientist with a strong, friendly personality, **wishing to work hard on provocative ideas, capable of supervising and coordinating several research projects.** He should like travel and work abroad. Conversational French an asset.

Send manuscript letter, C.V., photograph and names of 3 referees to *Box W3823 c/o Nature, 4 Little Essex Street, London WC2R 3LF.*

(W3823)A

LUDWIG INSTITUTE FOR CANCER RESEARCH
MONTREAL BRANCH

TUMOR BIOLOGIST

The Ludwig Institute has been newly established in Montreal to conduct basic and clinical research in conjunction with the Royal Victoria Hospital and thr McGill University Faculty of Medicine. Our program is directed toward understanding the relationship between normal organismal development and embryonal tumors.

We now seek an individual with research interests in the interactions of growth or differentiation factors and cellular oncogenes during mammalian development and tumorigenesis to head our Laboratory of Molecular Oncology. Applicants should have postdoctoral experience, a record of significant accomplishment in this area and an interest in appropriate collaborative interactions with our Laboratories of Molecular Genetics, Developmental Biology, Clinical Research and Cell Biology.

The appointment will be at LICR levels equivalent to assistant or associate professorship; corresponding academic appointment will be possible through McGill University. Support for salary, relocation, laboratory personnel, equipment and supplies will be provided. Preference will be given to qualified Canadian citizens or permanent residents.

Written applications including a curriculum vitae, names of three referees and a short synopsis of research accomplishments and directions should be received no later than June 1, 1987 by: W.K. Cavenee, Montreal Branch, Ludwig Institute for Cancer Research, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1. (NW558)A

PLANT BREEDING INSTITUTE

WATER RELATIONS PHYSIOLOGIST

required for approximately two years, supported by ODA funding, to undertake field and laboratory studies on the physiology of drought tolerance in barley. The work will involve observations and measurements on barley genotypes of diverse origin and the aim is to identify plant characteristics which confer adaptation to Mediterranean environments. The person appointed will be based in Cambridge, but working visits to ICARDA, Syria will be needed.

Candidates should have a first or upper second class honours degree in an appropriate biological subject, with at least two years' postgraduate experience in and a good knowledge of the techniques of plant water relations research.

Appointment will be at Higher Scientific Officer (£8,405 to £11,323 per annum). Applications with c.v. and names and addresses of three referees should be sent to the **Personnel Officer, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ,** quoting reference PHY/269 by 26th March 1987. Further particulars available on request.

The Institute is an Equal Opportunities Employer. (2196)A

Johns Hopkins University
School of Medicine

POSTDOCTORAL FELLOW IN IMMUNOGENETICS/ MOLECULAR BIOLOGY

with Ph.D. degree or M.D. (with research experience) to work on genetics of human immune response to allergens. Group with 15 years of experience in this area is now making a major thrust into the sequencing of HLA-D-region genes. Candidate **must** have background in recombinant DNA technology; experience in protein purification/characterization advantageous. Demonstrated research creativity essential. Send résumé, Curriculum vitae, copies of two publications and names of 3 to 4 referees to: **David G. Marsh, Ph.D., The Johns Hopkins University School of Medicine at The Good Samaritan Hospital, 5601 Loch Raven Boulevard, Baltimore, MD 21239.** *Affirmative Action/Equal Opportunity Employer.* (NW554)A

Team Leader
MICROBIOLOGY RESEARCH
Berkshire

ICI Plant Protection Division, based at our attractive, rural Jealott's Hill Research Station, is one of the forerunners in the expanding and vital area of crop protection. Due to our expansion programme and a recent promotion, we are seeking a PhD Microbiologist to give practical, laboratory based leadership to a small team involved in researching applications of biotechnology to both agrochemical and biological areas.

This challenging environment would suit a creative, innovative scientist with previous experience of biological control and natural products, ideally coupled with environmental awareness, experience of Mode of Action studies and a knowledge of genetics/ fermentation.

There will be plenty of scope to widen your responsibilities as scientific progress in this major project proceeds.

Competitive salary and benefits include membership of the Pension Fund and Profit Sharing Scheme.

Please apply in writing, quoting Ref. KAP/TO to: Joe Booth, Personnel Officer, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY.

Closing date for applications: 19th March 1987.

ICI is an equal opportunities employer.

(2240)A

ICI Plant
Protection
Division
The world harvests the benefits

PSYCHIATRIC GENETICS PROGRAM
epartment of Phsychiatry, Medical College of Virginia/
Virginia Commonwealth University

is recruiting a fulltime

RESEARCH SCIENTIST

terested in applying molecular genetic techniques to neuropsychiatric iseases. This person will join an active research team with a strong mphasis on quantitative genetic methods, with several major psychiatric enetic field studies currently underway. Responsibilities will include upervision of linkage analyses using RFLP's and establishment of immortalized cell lines. The candidate will be strongly encouraged to estab-sh his/her own research program in an area relevant to the molecular iology or neuropsychiatric diorders. Must have Ph.D. or equivalent and dvanced research training in molecular genetics. This will be a tenure rack position. Send CV and references to **Dr. K. Kendler, Depts. of Psychia-ry and Human Genetics, MCV, Box 710, Richmond, VA 23298.** *MCV/VCU is n EEO/AA Employer.* Women and minorities are encouraged to apply.

(NW502)A

POSTDOCTORAL POSITION

available July 1, 1987 or sooner to study the regulation of polyamine synthesis in plant cell cultures. Qualifications: Doctorate in Biology, Botany or Biochemistry. Experience in plant cell cultures, enzymology and/or recombinant DNA technology desirable. Appointment n annual increments for up to 3 years. Salary range is $17,010 — 9,040 depending on experience plus full benefits.

end curriculum vitae and three letters of reference (non-citizens nust include current visa status) before April 15, 1987 to:

Professor Subhash C. Minocha
Department of Botany & Plant Pathology
University of New Hampshire
Durham, New Hampshire 03824

UNH is an AA/EEO employer (NW562)A

AUSTRALIA
CSIRO
RESEARCH FELLOW
(Remote Sensing)
A$28,107 – A$41,339
MARINE LABORATORIES
DIVISION OF OCEANOGRAPHY
HOBART TASMANIA

GENERAL: The Division of Oceanography undertakes research into large and small scale physical and chemical oceanography, air-sea interaction and climate, currents and waves in the surrounding oceans. The Division operates a modern oceanographic research vessel FRANKLIN as a National Facility and has a satellite reception (L and S bands) and image processing facility which can relay images to the FRANKLIN. The Remote Sensing section is expanding to utilise airborne data with emphasis on ocean colour.

Satellite data from the Nimbus-7 Coastal Zone Color Scanner (CZCS) have shown up fine scale patterns in chlorophyll and suspended sediment that sometimes do not appear in thermal imagery. In coastal waters off Tasmania, suspended materials from terrestrial run-off complicate the spectral characteristics of ocean water.

DUTIES: As a member of the Remote Sensing group in the Division of Oceanography, the appointee will undertake a research and development program to apply ocean colour sensing to problems in oceanography. This will require research into the spectral bands best suited to determining the local circulation patterns and suspended substances. This research will be directed towards specifying instrumentation that could most usefully be flown on a future satellite system.

An initial task will be to develop a program of airborne remote sensing and to participate in the utilisation of a multichannel 'Daedalus' scanner and a spectrometer developed by the Division of Mineral Physics and Mineralogy. The appointee will have to modify existing equipment and possibly design new equipment. It will be necessary to collaborate with the Division of Mineral Physics and Mineralogy in the use of this equipment and with the Marine Laboratories in the use of analysis data. The appointee will have to fly with the equipment in the CSIRO F27 Research Aircraft.

LOCATION: For the first year the appointee will be located at the Division of Mineral Physics and Mineralogy at North Ryde, NSW. Some time thereafter the appointee may be transferred to the Marine Laboratories at Hobart.

QUALIFICATIONS: A PhD degree or equivalent qualification in applied physics, electro-optical engineering or other relevant discipline. A knowledge of digital data systems and data processing is required. Previous experience in this field would be advantageous, but is not required.

TENURE: A term of 3 years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal details, including details of qualifications and experience and the names of at least two professional referees and quoting reference No. A4358 should be directed to.

The Chief
Division of Oceanography
CSIRO Marine Laboratories
GPO Box 1538
HOBART, TASMANIA 7001
AUSTRALIA

by March 27, 1987.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W3836)A

HERIOT-WATT UNIVERSITY
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited from physicists or physical chemists for the above post to work with Dr. J. Pfab on an E. C. funded research project on the laser detection, analysis and spectroscopy of nitrogen oxide containing atmospheric pollutants in supersonic jets. The successful applicant will join a small group working in a well equipped laser laboratory in the Department of Chemistry.

Previous experience and a strong interest in gas phase techniques, spectroscopy or instrumentation will be advantageous. The appointment will be made for one year initially, but is extendable up to two and a half years.

Salary in the range £8,020 to £12,780 pa (under review). Please write (not phone) for application forms, returnable by March 30, 1987 to: the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX, quoting reference number: 13/87/N. (2204)A

ROYAL GREENWICH OBSERVATORY
LA PALMA, CANARY ISLANDS
ASTRONOMY GROUP LEADER

The Royal Greenwich Observatory has a vacancy for an experienced astronomer to lead the Astronomy Group on the island of La Palma, Canary Islands. The appointment will be fixed-term for about 3 years but may be extendable for up to 6 years by mutual agreement.

The group has responsibility for the scientific performance of the telescopes on La Palma which are operated for visitors from the British, Dutch and Spanish astronomical communities. The 1.0m Jacobus Kapteyn Telescope and 2.5m Isaac Newton Telescope are in full operation. The 4.2m William Herschel Telescope is at present under construction and a Starlink-type VAX computer for data reduction will be installed on La Palma this year. The group contains resident astronomers who carry out observations themselves, support the visitors and are responsible for the scientific performance of the astronomioal instruments. They compete for time on the La Palma telescopes; they will help commission the William Herschel Telescope.

The leader of this group must have maturity and be capable of getting the respect of his team and visitors of all three nationalities. He will have a record of outstanding achievement in astronomy and must be able to plan and direct work on observational astronomy.

The post is Grade 7 level, with a salary scale £14,318—£19,465 per annum.

Additional allowances are paid during overseas services, including the provision of free housing. Although the actual position depends on family and other circumstances, the salary is in effect tax free while resident on La Palma.

For further information and an application form, please contact Mrs J. Brooke, Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, East Sussex BN27 1RP. Tel: 0323-833171 ext. 3294.

The closing date for receipt of applications is 20th March 1987.

(2198)A

serc Royal Greenwich Observatory

UNIVERSITY OF WALES COLLEGE OF MEDICINE
DEPARTMENT OF MEDICINE (SECTION OF NEUROLOGY) AND DEPARTMENT OF MEDICAL BIOCHEMISTRY

MOLECULAR STUDIES IN MULTIPLE SCLEROSIS

Following the recent award of substantial funding from the Multiple Sclerosis Society, an existing group actively researching biochemical, immunological and applied aspects of multiple sclerosis is expanding and now wishes to appoint up to

4 NON-CLINICAL RESEARCH OFFICERS

at graduate or postdoctoral level for 3 years in the first instance. The work involves characterising the molecular basis of demyelination by defining intracellular consequences of cellular and humoral immune mediated damage in single cells using novel luminescent and immunological reagents.

Successful applicants will gain experience in tissue culture, image analysis, fluorescent cell sorting, derivation of T cell lines and clones, immunoassays and monoclonal antibody technology. Previous experience in some of these areas would be an advantage.

Salary will be on the scales for University Research and Analogous Staff Grade 1B or 1A (£7,055—£12,780 per annum, with substantial pay rise pending); starting point depending on experience.

Further particulars available from the **Registrar and Secretary (Personnel Office), University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN (Tel. No. 0222/755944 Ext. 2296)** to whom applications in the form of a full curriculum vitae and quoting Ref. No. M6/168 should be submitted three weeks from the appearance of this advertisement.

More details may be obtained from **Drs Alastair Compston, Tony Campbell, Paul Morgan or Chris Linington (0222/755944 Ext. 2835 or 2803).** (2163)A

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY (UNIVERSITY OF LONDON)
TWO POSTDOCTORAL RESEARCH ASSISTANTS

(1) MOLECULAR PLANT VIROLOGY. For a molecular analysis of cell-to-cell transport of virus particles in plants. The work will include use of bacterial expression vectors containing the cloned, virus transport protein gene, use of gold-labelled antibodies to detect the subcellular location of the transport protein and use of Ti plasmid vectors to obtain plants constitutively expressing the transport protein gene.

(2) MOLECULAR PLANT PATHOLOGY. For the construction of a restriction fragment length polymorphism (RFLP)-based linkage map of the genome of the fungal plant pathogen, *Phytophthora infestans*, causal agent of potato blight and is a collaborative venture with the University College of North Wales, Bangor. The twin objectives are to obtain RFLP markers to obtain linkage with genes for virulence and for use in population studies.

The appointments will be for three years. Initial salary up to £10,888 incl. LA., under review). Applications with c.v. and the names of two referees, as soon as possible and not later than 30 April 1987 to Professor K.W. Buck, Department of Pure and Applied Biology, Imperial College, London SW7 2BB.

(2188)A

THE OPEN UNIVERSITY
Faculty of Science
RESEARCH ASSISTANT

Applications are invited for the post of research assistant in our Oxford Research Unit. The postholder will work on a project investigating the role of the molecular mechanism of muscle contraction.

Applicants should have a degree in physics or equivalent qualifications. Experience in computing would be useful but is not essential.

The post is available from 1 April 1987 for a period of 36 months. Starting salary will be at the lower end of the IB scale for research and analogous staff: £7,055 - £9,495 pa (under review).

Application forms and further particulars are available from Mrs B Woodall (7034/3), Faculty of Science, Wimpey II, The Open University, Walton Hall, Milton Keynes, MK7 6AA, or Tel: Milton Keynes (0908) 653993: there is a 24 hour answering service on 653868.

Closing date for applications: 16 March. (2222)A

University of London
SCHOOL OF PHARMAC
LECTURESHIP IN PHARMACEUTICAI CHEMISTRY
(Specialist in Biochemistry

Applications are invited fr
graduates in pharmacy, bioch
istry or other appropriate subj
with strong interests in t
chemical applications in pharm
and related sciences. Suita
research areas include recept
macromolecular structure, enz
ology, metabolism, immunoche
istry, neurochemistry, molecu
and developmental biochemist
oncology and toxicology. Curr
programmes in the Departm
involve gene technology, mic
bial biochemistry, membrane p
teins, lipid metabolism, molecu
parasitology, synthetic medici
and peptide chemistry, biologi
NMR, mass spectroscopy, pl
sical and analytical chemistry.

The successful candidate will
expected to participate in teach
honours B Pharm and BSc (T
cology and Pharmacology)
grees.

Salary in the range £8,02
£15,700 p.a., currently un
review, plus £1,393 p.a. Lond
Allowance.

Applications including a f
curriculum vitae (6 copies),
scriptions of proposed resea
and names of two referees sho
be sent to the Assistant Secreta
School of Pharmacy, University
London, 29/39 Brunswick Squa
WC1N 1AX from whom furt
particulars may be obtaine
Interviews are likely to be h
towards the end of April.

(2191)A

THE INSTITUTE OF CANCER RESEARCH
TECHNICIAN

A technician is required to wo
with the Section of Pathology
the Institute of Cancer Resea
(Fulham Road Branch) to inve
igate genomic abnormalities
cancer. This will involve worki
with pathologists and molecul
biologists on methods for extra
tion and analysis of DNA fro
fresh human tissues and paraffi
wax embedded material. Son
knowledge of, or experience i
molecular biology techniques
preferred.

Salary in the range of £6,368-
£8,986 p.a. plus Outer Lond
Allowance of £677 p.a.

Applicants are advised that
the majority of the Institute
premises, smoking is prohibited.

Applications in duplicate wi
the names and addresses of tv
referees to be sent to the Perso
nel Officer, The Institute
Cancer Research, 17A Onslo
Gardens, London SW7 3A
quoting Ref. No. 2/87/T/N/61.

(2218)A

AFRC INSTITUTE FOR GRASSLAND ANIMAL PRODUCTION

IMMUNOLOGIST

uired at Shinfield, Reading to
a team of biochemists and
siologists investigating the
monal control of growth and
ation in farm species. The
licant will be expected to de-
op an innovative programme of
tegic research with immuno-
ical methods of modifying
mone action and the response
hormones. The objective is to
rease productive efficiency and
positive attitude to applied
earch is essential.

Areas of expertise relevant to
proposed research include:
uvant development; mono-
nal antibody production; syn-
tic peptides as immunogens;
i idiotypes; anti receptor anti-
ies.

The Institute has good facilities
immunoassay and cell culture.
as extensive holdings of sheep,
ats, cattle and pigs, as well as
ratory animal facilities.

QUALIFICATIONS: First or
per second class degree in bio-
mistry or a related science.
ur or more years of directly
evant research experience in
munology, with a PhD or equiv-
nt qualification, showing
idence of productivity and
ativity. Experience or know-
lge of endocrinological systems
uld be an advantage.

SALARY: Senior Scientific
ficer £10,558—£13,762. Unified
ade 7 £14,318—£19,465. There
a non-contributory superannua-
n scheme.

Applications with curriculum
ae and the names of three
erees to the Personnel Officer,
itute for Grassland and
nimal Production, Hurley,
aidenhead by 26 March 1987
oting S/D/49. (2189)A

NIVERSITY OF DURHAM

DEPARTMENT OF BOTANY

pplications are invited from suit-
ly qualified candidates for the
post of

ENIOR DEMONSTRATOR

tissue culture, tenable for at
ast three years, with a starting
ate from as soon as convenient.
uties will include lecturing and
search.

Initial salary in the range £7,055
-£9,495 p.a. (under review) on
rade 1A/B with superannuation.

Applications (3 copies) naming
ree referees (quoting reference
1), should be sent by 20 March
987 to the Registrar, Science
aboratories, South Road,
urham, DH1 3LE, from whom
rther particulars may be ob-
ained. (2219)A

THE AUSTRALIAN NATIONAL UNIVERSITY

MINERALOGY RESEARCH CENTRE

POSTDOCTORAL FELLOW/ RESEARCH FELLOW

Applications are invited from men and women with experience of or an interest in the use of transmission electron microscopy in the study of microstructures which influence the physical and chemical properties of geologically important materials. This (limited tenure) position would be of interest to materials scientists, metallurgists, solid state physicists and chemists, mineralogists and geologists. The work is in the Mineralogy Research Centre, which consists of groups from the Research Schools of Chemistry and Earth Sciences and the Department of Geology, and is equipped with three microscopes — Philips 430, JEOL 200CX and JEOL 100CX — as well as high-temperature and high-pressure facilities.

Depending on the level of appointment, salary will be within the range: Postdoctoral Fellow $A24,013—27,507 p.a.; Research Fellow $A27,859—36,600 p.a. Reasonable travel expenses. Maternity leave. Superannuation. There is no application form. Applicants should quote Ref. No. RC.30.1.1. and supply to the Registrar, Australian National University, G.P.O. Box 4, Canberra, A.C.T. 2601, Australia, by 15 May 1987, a curriculum vitae, list of publications, statement of research interests, two passport-sized photographs, names and addresses of three academic referees, and probable date on which the appointment could be taken up.

Further particulars on the appointment may be obtained on request from the University or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. The University is an equal opportunity employer.

(W3849)A

SENIOR MLSO

required. Duties to include the investigation of abnormalities in the spermatoza of patients with retinal diseases. The techniques will include microscopic, photographic and 2D electrophoresis. The successful applicant will be required to interact with volunteer patients.

Three-year appointment; starting salary £9,727 p.a. (including London Allowance). Applications to Professor G.B. Arden, Department of Clinical Opthalmology, Institute of Ophthalmology, Judd Street, London WC1H 9QS, giving details of qualifications and experience, and day-time telephone number. (2168)A

AUSTRALIA

CSIRO

RESEARCH FELLOW

MOLECULAR BIOLOGIST

A$28,107 – A$34,681

DIVISION OF TROPICAL ANIMAL SCIENCE

LONG POCKET LABORATORIES

INDOOROOPILLY QLD

GENERAL: The Division of Tropical Animal Science undertakes research activities on the improvement of animal health and production, particularly cattle production, in tropical Australia. It has programs on breeding and management, disease and parasite control and nutrition.

A major area of research being conducted at the Division's Long Pocket Laboratories is the development of experienced vaccines against diseases. The Division has groups undertaking immunological, biochemical and molecular biology studies of the antigens of disease agents. A number of molecular biologists are being appointed to assist these studies. One of these programs is aimed at alleviating the potentially fatal toxicosis, tick paralysis, caused by the Australian paralysis tick *Ixodes holocyclus* in domestic livestock and pets.

The tick paralysis program has reached the stage where the feasibility of vaccinating animals against the crude toxin has been demonstrated and antigen purification and characterisation is well advanced.

DUTIES: To undertake collaborative research work to produce the *Ixodes holocyclus* antigen molecule in whole or part by recombinant DNA methods.

QUALIFICATIONS: A PhD degree or equivalent qualifications in the field of recombinant DNA technology.

TENURE: A term of three years with Australian Government superannuation benefits available.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting Ref No: M7800, should be directed to:

The Chief
CSIRO Division of Tropical Animal Science
Private Bag No 3
INDOOROOPILLY QLD 4068
by March 27, 1987.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W3837)A

THE UNIVERSITY OF SYDNEY

Director — Ref. No. 7/02

Postgraduate Foundation in Veterinary Science

The retirement of the present Director, Tom Hungerford, OBE, has created an exciting opportunity for a person who enjoys challenge and can lead the Foundation in a new era.

The Postgraduate Foundation in Veterinary Science and Postgraduate Committee in Veterinary Science in the University of Sydney is concerned with encouraging and providing continuing education for veterinary graduates of all faculties in Australia and New Zealand. It is the most far reaching and highly regarded organisation of its kind in the world.

The successful applicant should have a degree in veterinary science and experience in practice. He or she will have the personal presence, maturity and persuasive approach necessary to gain the acceptance of the 5,000 practitioners who draw on the services provided by the Foundation. The rewarding goals include:

- Developing and implementing a plan of educational events and publications.
- Leading a dedicated staff operating from the Sydney offices
- Applying EDP technology to streamline information storage, retrieval and indexing.
- Extending the global network to strengthen Foundation links.

The salary will be negotiated according to qualifications and experience.

Further details may be obtained from Professor M.J. Edwards, Chairman, Postgraduate Committee in Veterinary Science, University of Sydney, NSW 2006, Australia.

Applications, quoting reference no., including curriculum vitae and the names and addresses of three referees to the Registrar, University of Sydney, NSW 2006, Australia, by 15 April 1987. (W3852)A

UNIVERSITY OF QUEENSLAND

Equal Opportunity in Employment is University Policy

CHAIR OF MOLECULAR BIOLOGY

Applications are invited for the newly established Chair of Molecular Biology within the Department of Biochemistry. Applicants must present evidence of outstanding research achievement in the field of molecular biology, and ability to collaborate with other scientists in the application of molecular biology to research in other areas of biochemistry and biology. Future plans give high priority to the establishment of a Centre for Molecular Biology and Biotechnology. Current areas of research in the Department include enzymology, mammalian and plant molecular biology, medical Current areas of research in the Department include enzymology, mammalian and plant molecular biology, medical biochemistry, neurochemistry, membrane biochemistry, biochemical pharmacology and physical biochemistry.

The appointee will be responsible to the Head of the Department of Biochemistry for producing academic leadership in undergraduate and postgraduate teaching and reasearch in molecular biology and related fields. Courses are currently offered in the Faculties of Dentistry, Medicine, Science and Veterinary Science. A Master of Scientific Studies in the field of Molecular Biology will be offered for the first time in 1987. Participation in the general administration of the Department and the University will also be expected.

Salary per annum: $A58,348
Closing date: 15 April 1987. Reference No. 06987

DIRECTOR, ELECTRON MICROSCOPE CENTRE

The appointee will have qualifications, experience and personal qualities which will allow leadership over the range of biological, physical and biochemical applications of electron microscopy, as well as high expertise in at least one area. Initial appointment will be for a fixed term of five years, to Senior Administrative/Professional staff Class 2.

Salary per annum: $A44,669 — 49,572.
Applications close 30 March 1987. Reference No. 08987

LECTURER/SENIOR LECTURER — ANATOMY

The appointee will contribute to the teaching of topographical anatomy to medical and paramedical students. Applicants should have demonstrated a research capability in an area of health sciences or biology. Preference will be given to medical graduates with teaching competency in neuroanatomy.

Salary per annum: Lecturer $A27,859 — $36,600
Senior Lecturer: $A37,381 — $34,568
Applications close 30 March 1987. Reference No. 06787

LECTURER (TENURABLE) — MATHEMATICS

The appointee must have a Ph.D. in mathematics or mathematical physics. He or she will be expected to teach large engineering and sciences classes. Some evidence of ability in this area is most desirable. While we welcome applicants with research interests in areas of mathematics currently pursued within the Department, we particularly welcome persons with a research record in any of the following: Control theory/robotics, engineering mathematics, mathematical logic, number theory, quantum mechanics. An appointment may be made at an intermediate point of the lecturing scale.

Salary per annum: $A27,859 — $36,600
Applications close 10 April 1987. Reference No. 07487

Additional information and application forms are available from the Secretary-General, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W3853)A

THE INSTITUTE OF CANCER RESEARCH (UNIVERSITY OF LONDON)

POSTDOCTORAL RESEARCH SCIENTIST: MASS SPECTROMETRY/ DRUG METABOLISM

Applications are invited for the above vacancy within the Drug Development Section at the Institute's laboratories in Sutton, Surrey, from persons having practical experience of the application of mass spectrometry in drug metabolism or other biomedically orientated studies. A good knowledge of NMR spectroscopy would also be an advantage. The successful candidate will be responsible for the characterisation of drug metabolites as part of a multidisciplinary team concerned with the development of novel chemotherapeutic agents.

The appointment is for three years. Salary in the range of £8,020 — £11,790 p.a. plus London Allowance of £1,297.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications, including a full curriculum vitae and the names and addresses of at least two referees should be sent in duplicate to The Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting Ref. No: 2/87/S/NA/60. (2181)A

CHARING CROSS AND WESTMINSTER MEDICAL SCHOOL (UNIVERSITY OF LONDON)

DEPARTMENT OF MEDICINE

RESEARCH ASSISTANT

Applications are invited for the above post, funded by the Medical Research Council for two years, to investigate the interaction between lung surface components and cultured lung cells with particular respect to the development of cigarette smoke-induced pulmonary emphysema and connective tissue turnover. The applicant must have some experience of tissue culture and cytochemistry, while knowledge of biochemical and immunochemical analytical techniques would be an advantage.

Salary not less than £8,020 p.a. plus £1,393 p.a. London Allowance.

Applications, including curriculum vitae and the names and addresses of two referees to The Secretary, Charing Cross and Westminster Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP, to be submitted within three weeks of the appearance of this advertisement. (2178)A

ROYAL GREENWIC OBSERVATORY

ASTRONOMER/ COMPUTER SCIENTIS

There is a vacancy at the F Greenwich Observatory fo observational astronomer/ puter scientist to head the dedicated team (3-4 staff) pro ing data from the ESA PARCOS satellite mission. candidate should have an e lished research reputation Galactic astronomy, and subs tial programming experience. suite of programmes curre under development is FORTRAN for a DEC V microcomputer; the final sys will probably be run on a dec ted MicroVAX. The satellite h proposed launch date of July 1 and the mission will end 3 y after launch. The position is on high responsibility: HIPPARC is a "cornerstone" ESA miss and the RGO data-proce consists of the primary reduc of the raw photon counts and c attitude reconstruction. The cessful candidate will manage organize the development of necessary software, specify hardware requirements, and li and arrange meetings with ot international groups involved the project.

During the mission he supervise the data processing the quality control. Subsequen the mission the astronomer continue the analysis of the d through to preparation of the fi catalogue and the derivation scientific results. A prime resp sibility during this phase will be ensure that the data are f exploited by UK astronomers have made proposals for mission.

Applicants should norma have obtained a 1st or 2nd cl honours degree together w doctoral degree in a scientific s ject or an equivalent qualificati but other candidates who produce evidence of high prof sional attainment may be acce able. Every candidate should ha had at least 4 years' appropri experience after qualifying.

The post is graded at Seni Scientific Officer level with salary £10,558—£13,762 p annum. Candidates should no that SERC has announced intention of resiting RGO at Ca bridge by about 1990, and i likely that the HIPPARCOS p ject will move to Cambridge w in advance of this.

Further information and app cation forms are available fro Mrs J. Brooke, Royal Greenwi Observatory, Herstmonce Castle, Hailsham, East Suss BN27 1RP, telephone 032 833171 ext 3294, to whom applic tions should be returned by 20 March 1987. (2197)A

›ostdoctoral Positions

ɩearch involves bio-
ɩmical and electrophysio-
ical studies of the role of
ɩsphoinositide metab-
ɩm in phototransduction.
chemist position avail-
e immediately, physio-
ist position September
37. Send curriculum vitae
ɩ three letters of reference
**Ɔr Alan Fein, Marine Bio-
ical Laboratory, Woods
le, MA 02543** no later than
ril 1, 1987. *An Affirmative
ion/Equal Opportunity
ɩployer.* (NW538)A

›OSTDOCTORAL POSITION

ailable immediately to in-
stigate the mechanism of
ion of insulin and the hor-
ɩnal control of protein
osphorylation in skeletal
ɩscle and fat cells. Salary
7,000—$22,000 per year
pending on experience.
nd resume to: **Dr John C.
wrence, Department of
armacology, Washington
iversity School of Medicine,
0 South Euclid, St. Louis,
ↄ 63110.** (NW474)A

Food Microbiologist

SHELF-LIFE RESEARCH

Blackfriars **up to £8,500**

At Sainsbury's, the U.K.'s leading food retailer, we go to considerable lengths to maintain our reputation for quality and safety. One of the first steps, which takes place in the modern research laboratory at our Blackfriars Head Office, is to carefully establish the shelf-life of every new product line.

Since our range of both own brand and branded products is growing at an ever-increasing pace, we are now looking for an additional food microbiologist to assist us in this vital task.

Aged in your mid to late 20's, you should have 3 to 4 years' experience in a food laboratory and strong communication and inter-personal skills. Familiarity with computerised systems would be an added advantage.

In return, we offer a salary of up to £8,500 plus a wide range of benefits including interest free season ticket loan, profit sharing and share option schemes after qualifying periods and subsidised restaurant facilities.

If you're looking for more independence, more responsibility and an opportunity to use your skills to the full, please write or telephone for an application form to Clare Spalding, Recruitment Officer, J Sainsbury plc, Wakefield House, Stamford Street, London SE1 9LL. (2239)A

SAINSBURY'S

POSTDOCTORAL POSITIONS

ɩvailable immediately with the National Heart, Lung, and Blood nstitute (NHLBI) to study the biochemistry and physiology of heat-shock proteins in yeast. The focus of the laboratory is on the role of he 70 kDa heat shock proteins in modulating the organization of ɩrotein complexes in yeast and the relationship of those heat shock ɩroteins to a homologous ATPase involved in the dissolution of ɩrotein complexes during endocytosis in mammalian cells. Salary starts at $20,000/year. Send curriculum vitae and three letters of ecommendation to: **Dr Evan Eisenberg, Section on Cellular Physiɩlogy, Laboratory of Cell Biology, NHLBI, National Institutes of ɩealth, 9000 Rockville Pike, Building 3, Room B1-22, Bethesda, Maryland 20892.** *An Equal Opportunity Employer.* (NW540)A

MOLECULAR VIROLOGIST

The Department of Medical Microbiology and Immunology, The Ohio State University Medical Center, invites applications for a tenure track faculty position at the rank of Assistant Professor. Applicants must have a strong background in molecular virology, emphasis on herpesviruses (Epstein–Barr virus in particular). Qualified applicants should arrange to have curriculum vitae and 3 letters of recommendation sent to: **Ronald Glaser, PhD, Professor and Chairman, Department of Medical Microbiology and Immunology, The Ohio State University College of Medicine, 333 West 10th Avenue, Columbus, Ohio 43210.** Deadline for completed applications is March 20, 1987. *The Ohio State University is an Equal Opportunity, Affirmative Action Employer.*
(NW529)A

UNIVERSITY OF MARYLAND
SCHOOL OF MEDICINE
Department of Biological Chemistry

POSTDOCTORAL RESEARCH ASSOCIATE

Position is available to study molecular mechanisms of action of presynaptic protein neurotoxins. Research involves receptor binding, transmitter release, and second messenger studies using cultured neural cells. Background in biochemistry, cell biology, or pharmacology is essential with a commitment to training in biochemical approaches. Salary negotiable.

Applications with CV and the names of three references should be sent to: Dr Terry B. Rogers, University of Maryland, School of Medicine, 660 W. Redwood St., Baltimore, MD, 21201. Equal Opportunity Employer. (NW563)A

POSTDOCTORAL POSITION

now available in biochemistry. We are an established group in the area of membrane structure and function, magnetic resonance spectroscopy and biophysics, with interests in signal transduction, protein kinases, phospholipase c, and molecular basis of tumor promotion. We are looking for a colleague with mutual interests and a strong background in biochemistry. Cloning experience helpful. Send vitae and names and addresses of three references to: **Dr O. Hayes Griffith, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403** by April 15, 1987. Telephone: (503) 686-4634. *An Equal Opportunity/Affirmative Action Employer.*
(NW528)A

Biosensor Breakthrough

Molecular Devices Corporation patented revolutionary silicon sensor technology last May. Our proprietary approach allows on-site analyses of ions, enzymes, drugs, proteins, and bacteria on the same sensor within minutes.

Microbiologist

We are seeking a PhD or MS Microbiologist to investigate and develop a proprietary system combining chemistry and instrumentation for the detection of bacterial and viral pathogens. Three-plus years' experience with generation and application of monoclonal antibodies to diagnostic procedures, as well as strong clinical microbiology background is desired.

To apply, please send your résumé to Human Resources, Molecular Devices Corporation, 3180 Porter Drive, Palo Alto, CA 94304. An equal opportunity employer.

(NW546)A

Molecular Devices

AFRC INSTITUTE OF FOOD RESEARCH READING LABORATORY

HEAD OF LABORATORY

Applications are invited for this post which has become available as the result of the amalgamation of the Council's three food research Institutes at Bristol, Norwich and Reading and the resignation of Professor M.I. Gurr to take up a post with the Milk Marketing Board.

The Reading Laboratory undertakes applied research relating primarily to quality aspects of milk, milk products, oils and fats, beverages and confectionery, as well as the nutritional quality of foods.

The Head of the Laboratory will be responsible for the day to day operation of the Laboratory and will be accountable to the Director of Food Research.

However, the primary responsibility of the successful candidate will be the scientific management of a substantial part of the Institute's programme of research in the general area of the safety and quality of foods within the three food-related laboratories of the Institute. The post is primarily concerned with programme management; and important role will be coordination of the appropriate area of research across the three Laboratories.

The Head of Laboratory will be employed by the AFRC but he/she will have the status of Professor in the University of Reading and be a member of the University Senate. Candidates should have qualified in food science or the related underlying scientific disciplines and have a distinguished record of research, together with experience in the management of research.

AFRC

This is a Grade 5 post with a salary in the range £23,730 to £27,065. Requests for further particulars and application forms should be sent to the Secretary, AFRC Institute of Food Research, Shinfield, Reading RG2 9AT. Closing date: 20 March 1987. (2202)A

MEDICAL RESEARCH COUNCIL

LABORATORY OF MOLECULAR BIOLOGY

RESEARCH ASSISTANT

Applications are invited for a Research Assistant with at least three years' research experience to work on immunoglobulin genes and oncogenes in transgenic mice. Experience in recombinant DNA technology, tissue culture or animal handling would be an advantage but not essential. Candidates should have an honours or higher degree or equivalent in an appropriate subject.

Further information is available from Dr M. Neuberger or Dr T.H. Rabbitts, Cambridge (0223) 248011.

The post is available for three years and the salary will be in the range £8,020—£12,280.

Applications — including a full c.v. and the names of two professional referees — should be sent by 12th March quoting reference PNAC/5231 to: The Personnel Officer, Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH.

The Medical Research Council is an equal opportunity employer.

(2177)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (UNIVERSITY OF LONDON)

DEPARTMENT OF CHEMICAL PATHOLOGY

Applications are invited for the post of

SENIOR LECTURER (Non-Clinical)

in the Department of Chemical Pathology. The person appointed will be experienced in peptide synthesis and analysis, and will be expected to direct a research programme relevant to the biochemistry/molecular biology of human disease complementary to research programmes already established in the School and Department, which include the molecular biology, chemistry and pathophysiology of polypeptide hormones; clinical enzymology; and general aspects of molecular biology.

Appointment to the post will be made at an appropriate point on the Senior Lecturer (Non-clinical) scale. Potential applicants wishing to discuss the post should contact Professor I. MacIntyre (01-740 3227). Further particulars are available from the Deputy Secretary, London W12 0HS (01-740 3203). Applications (six copies of full c.v. plus names of three referees) should reach the Deputy Secretary at the above address by 2nd April 1987. Reference AC/3.

(2221)A

POSTDOCTORAL POSITION IN MOLECU BIOLOGY

Paris, France

A postdoctoral position is able from July 1987 to co research on the human eryth specific enzyme 2,3-bisphos glycerate mutase (2,3-BP recently cloned in this laborat

The successful applicant join a group studying the s tural and transcriptional orga tion of the gene, as well as the chemistry of the enzyme using directed mutagenesis. Candi should have experience in m ular genetics and/or in pr design.

The level of appointment salary will be commensurate experience and qualifica (salary range 8,000—10,00 month). Applicants should sess a PhD or equivalent deg French citizens are exclu Position initially available f July 1987 for 1 year and renew for up to two years.

Applications including cu lum vitae, reprints of scie publications, and the names of referees should be sent Dr Michel Cohen-Solal, U Inserm-U91, Hopital H Mondor, 94010 Créteil (Fran Tel.: (33-1) 48 99 27 08.

(W3842).

THE INSTITUTE OF CANCER RESEARC (UNIVERSITY OF LONDO

POSTDOCTORAL FELL

A three year postdoctoral pos is available to study the trans tional control of gene expres during spermatogenesis in Developmental Genetics T (Head: K. Willison) at the Che Beatty Laboratories, Ful Road, London.

Currently we are develop transcriptional assays for hap expressed genes in isolated g cells, transgenic mice and *in vi* The successful applicant co decide which topic interested h her most but is expected to be of this team effort. The posit would suit a PhD graduate Molecular Biology or chemistry.

Salary in the range of £9,31 £13,087 all inclusive.

Applicants are advised that the majority of the Institu premises smoking is prohibited

To apply for the post ple forward a curriculum vitae duplicate with the names addresses of two referees to Personnel Officer, The Institute Cancer Research, 17A Onsl Gardens, London SW7 3A quoting Ref. No: 2/87/S/N/62.

(2183)A

THE UNIVERSITY OF SYDNEY

LECTURESHIP (TENURABLE) IN HYSICAL CHEMISTRY

Reference no. 6/10

School of Chemistry

plicants should possess a PhD ferably in physical chemistry or mical physics. Duties will inde undergraduate teaching at evels, postgraduate lecturing, prosecution of an active earch programme and the ervision of postgraduate earch students. Applications invited from all qualified sons irrespective of their field interest in Physical Chemistry; ference may be given to applits with a proven research ord in materials science or sure science (including catalysis). e appointment will commence 1 August, 1987.

Appointments to lectureships capable of leading to tenure d are usually probationary for ee years.

Salary: Commencing salary in range $A27,859—$A31,605 r annum (with progression to 36,600 per annum.

Applications, quoting reference ., including curriculum vitae, t of publications and the names d addresses of three referees, to Registrar, University of Sydy, NSW 2006, Australia, by 3 ril 1987.

The University reserves the ht not to proceed with any pointment for financial or other asons.

Further information available m Association of Commonealth Universities (Appts), 36 rdon Square, London WC1H F. (W3843)A

CENTRAL BIRMINGHAM HEALTH AUTHORITY

BIRMINGHAM ND MIDLAND HOSPITAL FOR WOMEN

NDOCRINOLOGY DEPARTMENT

THREE BIOCHEMIST/ SCIENTIFIC OFFICERS

quired for a maximum of two ears to work in Reproductive ndocrinology (research funded).

Two posts will be involved with evelopment of monoclonal antidies and one post with the purication of pituitary hormones.

Salary Scale: Basic Grade Proationer/Post Probationer accordg to experience.

Application forms from: Personnel Department, Birmingham aternity Hospital, QEMC, dgbaston, Birmingham B15 TG. Informal enquiries to Mr W. obinson (021 772 1101 ext 296). losing date: 12th March, 1987. (2164)A

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT, CHILTON AND

CENTRAL ELECTRICITY GENERATING BOARD

BERKELEY NUCLEAR LABORATORIES, BERKELEY

CELL BIOLOGISTS/ RADIATION-ONCOGENESIS

(RE-ADVERTISEMENT)

Applications are invited for two short-term Non Clinical Scientific Posts (tenable for 3 years) created through the initiation of a joint MRC/CEGB study of radiation-induced oncogenic transformation in cultured mammalian cells.

Post A based at MRC, Chilton: To study oncogenic transformation in cultured haemopoietic cell lines using both cellular and molecular techniques (experience in cell culture techniques necessary, training in recombinant DNA techniques available).

Post B based at CEGB, Berkeley: To study the quantitative aspects of oncogenic transformation in established rodent fibroblast culture systems (basic experience in cell culture techniques desirable).

For post A candidates of postdoctoral or equivalent status will be preferred, for post B candidates with either PhD or MSc qualifications will be considered.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff and application forms may be obtained from the Personnel Officer, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, with whom applications including a full CV and the names of two professional referees should be lodged. Previous applicants need not apply. For futher information please telephone Dr Roger Cox on Abingdon (0235) 834393. The Council is an equal opportunity employer. Closing date: 23rd March 1987. (2228)A

POSTDOCTORAL RESEARCH ASSISTANTS

interested in working on the biochemistry of parasitic protozoa (African Trypanosomes, Leishmania) and their interaction with the vertebrate host. Candidates should have experience in biochemical, molecular genetic and/or immunological techniques. Salary according to BAT III/II (DM 45,500—52,000 p.a.).

Contracts will be initially for two years with the possibility of extension for further three years. Prof. Dr P. Overath, Max-Planck-Institut für Biologie, Correnstraße 38, 7400 Tübingen, FRG. (W3840)A

AUSTRALIA

CSIRO

RESEARCH SCIENTISTS

(2 POSITIONS)

A$28,107 – A$41,339

SPACE RESEARCH AND SATELLITE REMOTE SENSING

DIVISION OF ATMOSPHERIC RESEARCH

ASPENDALE VIC

GENERAL: The Division has for some years been developing a major system (CSIDA) for reception and analysis of data from the operational meteorological satellites (AVHRR data in particular). The Division is also involved in collaboration with laboratories in the United Kingdom in the development and scientific applications of the ATSR instrument to be flown on the ERS 1 satellite in 1989. In addition it is performing feasibility studies for new satellite instruments which are potential candidates for future space platforms (e.g. an imager/sounder for a geostationary satellite, an instrument for remotely sensing surface pressure from a polar-orbitor, and an on-board data compression device for studies of cloud distribution).

Space Research is a priority area within CSIRO and the Division is currently strengthening the resources devoted to the above activities.

1. SCIENTIST — SATELLITE REMOTE SENSING — Reference Number A1830

DUTIES: In association with other Divisional scientists — in general to pursue research associated with the developing international ATSR programme and with the rapidly expanding capabilities of the CSIDA system. In particular to pursue research into applications of ATSR, AVHRR and associated satellite data — mainly for purposes of investigating atmospheric phenomena, but also for monitoring surface parameters such as vegetation indices, sea surface temperature etc.

QUALIFICATIONS: A PhD degree or equivalent qualifications in physics or applied mathematics, with relevant experience in atmospheric science and remote sensing and (preferably) some experience in satellite data handling.

2. SCIENTIST/ENGINEER IN SATELLITE INSTRUMENTATION

Reference Number A1825

DUTIES: In association with other Divisional scientists, to undertake research into the engineering design and development of prototype satellite hardware, and in the logistic planning of subsequent phases of development of that hardware. The appointee would need to interact with local and overseas aerospace companies to achieve the maximum possible technology transfer to Australia.

QUALIFICATIONS: A PhD degree or equivalent qualifications in Physics or Engineering and experience in the overall process of satellite instrument development. Demonstrated ability to interact well with industry.

Both Positions:

TENURE: Indefinite with Australian Government superannuation benefits available. However, applicants interested in a term appointment of three to five years will be considered.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience, the names of at least two professional referees and quoting the appropriate reference number should be directed to:

The Chief
CSIRO Division of Atmospheric Research
Private Bag No. 1
MORDIALLOC VIC 3195
AUSTRALIA

by April 3, 1987.

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W3838)A

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nature

the widest international selection of jobs in science — EVERY WEEK

TRANSPLANTATION IMMUNOLOGIST

The Cleveland Clinic Foundation invites applications for a full-time position involving a primary appointment in the Department of Immunology & Cancer and a joint clinical appointment. Interested applicants must have an MD and/or PhD degree with considerable postdoctoral experience in basic transplantation immunology. Candidates must be established investigators with evidence of high quality independent research, and a consistent history of external funding support. Essential qualities of the successful applicant are the ability to develop a research program bridging basic laboratory investigation with relevant problems in transplantation science, and a willingness to interact with basic scientists as well as transplant clinicians. Please submit a letter of application, curriculum vitae, and research interests to:

Andrew C. Novick, M.D.
Chairman, Department of Urology

THE CLEVELAND CLINIC FOUNDATION
9500 Euclid Avenue Cleveland Ohio 44106

The Cleveland Clinic Foundation is an Equal Opportunity/Affirmative Action Employer.

(NW473)A

Sandoz Ltd., a major international chemical company headquartered in Switzerland, wishes to appoint a well-qualified

Organic / Bio-organic Chemist with computer experience

to a post in the Molecular Modelling/Drug Design Group in Basle.

Candidates should have a Ph.D. in organic chemistry and experience in applying molecular graphics, molecular mechanics and quantum chemistry to structure/activity problems and to enzyme/inhibitor interactions. A broad background in medicinal chemistry and general pharmacology is required, together with experience of computer programming.

The Molecular Modelling Group is at present using an E+S–PS 340 graphic system connected to a dedicated VAX (VMS).

Applications, including CV, should be sent to:

SANDOZ

Sandoz Ltd., Personnel Department, Ref. 7104
attn: Mr. J. Brunner.
CH-4002 Basle

(W3835)A

UNIVERSITY OF STOCKHOLM
WENNER-GREN INSTITUTE
Department of Ultrastructure Research

ELECTRON MICROSCOPE

Applications are invited for a 2+2 year postdoctoral position to work within the field of biological ultrastructure. The position includes some teaching and administration in an EM-laboratory, which is well equipped for TEM including X-ray microanalysis and cryotechniques. Experience in at least some of these fields is desirable. Salary within the range of £12,000 - £15,000 per annum.

Applications with a curriculum vitae and a statement of scientific achievements and intentions should be sent to: **The Registrar, Stockholm University, S-106 91 Stockholm, Sweden,** Ref No: 109/87 before March 23. Names and addresses of two referees are recommended. Further information is available from Dr. B. Afzelius, Wenner-Gren Institute, University of Stockholm, 106 91 Stockholm. Tel No: 8 - 16 40 91.

(W3825)A

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF GEOLOGICAL SCIENCES

TEMPORARY LECTURER IN STRUCTURAL GEOLOGY

Applications are invited for the post of Temporary Lecturer in structural geology arising from externally-funded research leave for Dr J.S. Watterson. Duties include the teaching and assessment of structural geology to undergraduates at all levels, fieldwork instruction and tutorials in general geology. The successful applicant will also be expected to contribute to the research activities of the department by developing his/her research interests.

Candidates should have completed, or virtually completed, their PhD Post-doctoral experience an advantage, but not essential. The appointment is for two years, commencing September 1st, 1987.

The initial salary will be on the scale £8,020—£9,495 per annum (under review).

Applications (three copies), together with the names of three referees, should be received not later than April 20th, 1987 by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/364/N. (2185)A

MEDICAL RESEARC COUNCIL
LABORATORY OF MOLECUL BIOLOGY

POSTDOCTORAL SCIENTIST

Applications are invited postdoctoral scientists to with Dr T. Creighton on the synthesis, proteolytic process and folding of the polypep precursors of two small eukar proteins. The main emphasi the research is on protein struc and folding, but it currently primarily recombinant DNA t nology. It is essential that ap cants have experience in ei protein chemistry or gen engineering and an interest both. Preference will be give applicants who are able to c mence their duties shortly.

Further information on the ject is available from Dr Creighton, Cambridge (02 248011.

The post is available for 3 y and the salary will be in the r of £9,495—£15,700 p.a.

Applications, including a c.v., a short statement of relev experience, and the names of professional referees, should sent by 12 March 1987 quot reference SS/2338R to: The P sonnel Officer, MRC Laborat of Molecular Biology, Hills Ro Cambridge CB2 2QH. T Medical Research Council is Equal Opportunity Employer.

(2195)A

THE SCHOOL OF PHARMACY THE UNIVERSITY OF LONDON
DEPARTMENT OF PHARMACEUTICAL CHEMISTI

POSTDOCTORAL RESEARCH ASSISTAN

Applications are invited for a po doctoral research assista funded by the WHO Onc cerciasis Chemotherapy Proje on the study of the carbohydr metabolism of nematodes as f lowed by HPLC, NMR and ot techniques, with the aim of desig ing and testing new antifila agents.

The appointment, which available immediately, is initi for a twelve months fixed but extension of funding will sought. Initial salary is £9,413 p. including London Allowan Applications with names of t referees and two copies of c.v. Personnel Secretary, The Sch of Pharmacy, 29/39 Brunswi Square, London WC1N 1A Further information may be tained from Dr J.W. Powell or R.A. Watt, Department of Ph maceutical Chemistry. Telepho 01-837-7651. (2172)A

Institute of Marine Studies

Reader in Oceanography

£14,784–£18,588
(Salary under review)
(Starting salary dependent upon qualifications and experience)

We wish to appoint a physical oceanographer to this senior research position in the newly-created Institute of Marine Studies. Candidates will probably possess a PhD in an appropriate discipline and have a substantial national and international reputation in coastal and shelf oceanography. Experience of sediment transport and the benthic boundary layer would be an advantage. The successful candidate would be expected to collaborate closely with other marine research in the Polytechnic and with the NERC (IMER, MBA) research institutes in Plymouth.

Application forms — to be returned by Friday 20th March 1987 — and further details are available from The Personnel Department, Plymouth Polytechnic, at the address below or by telephoning (0752) 264639. (2227)A

Plymouth Polytechnic
Drake Circus, Plymouth
Devon PL4 8AA

UNIVERSITY OF OXFORD

Department of Paediatrics
John Radcliffe Hospital
Headington, Oxford OX3 9DU

An experienced postdoctoral esearch fellow is required to oin a group investigating the general basis of bacterial virulence expression. The Department has a strong interest in the biological basis of infectious diseases and the successful applicant must have a research background in molecular genetics and/or biochemistry.

The position is funded by the Medical Research Council until 31 March, 1990, in the first instance. The salary is on Scale RSII (£11,790 - £15,700) currently under review.

Applications in writing with a full curriculum vitae including the names and addresses of 3 referees should be sent to: **Professor E R Moxon, Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU.** Closing date: 16 March 1987. (2187)A

UNIVERSITY OF OXFORD

DEPARTMENT OF PAEDIATRICS
JOHN RADCLIFFE HOSPITAL
Headington, Oxford OX3 9DU

A postdoctoral research fellow is required to study the biochemical basis of bacterial virulence expression. The successful applicant will be required to work on the biosynthetic pathways of capsular and lipopolysaccharide antigens and to provide biochemical expertise to complement the ongoing genetic analysis of *Haemophilus influenzae*.

The salary is on the Scale RS1A (£8,020 — £10,375) currently under review. The position, funded by the Medical Research Council, is tenable until 31 March, 1990 in the first instance.

Applications in writing with a full curriculum vitae including the names and addresses of 2 referees should be sent to: **Professor E.R. Moxon, Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford OX3 9DU.**

Closing date: 16 March 1987. (2186)A

MICROBIAL ECOLOGIST

Postdoctoral position for at least one year, available immediately. Biological control-oriented, interdisciplinary project involves isolation, identification and determination of the ecological role of microbial colonists of aquatic weeds. Experience in microbial ecology, bacteriology, mycology, or plant pathology. Send résumé, transcripts, and names, addresses and telephone numbers of at least three references to: **Dr R.F. Harris, Department of Soil Science, University of Wisconsin, Madison, WI 53706.** *An Equal Opportunity Employer.* (NW543)A

POSTDOCTORAL RESEARCH ASSOCIATE

Postdoctoral Research Associate position available in August 1987 to study migration and function of activated T lymphocytes in the intestine. Candidate should have experience in techniques for studying lymphoid cell migration, labelling and *in situ* identification. Interested applicants should send a curriculum vitae and the names and addresses of three referees to: **Dr R.G. Bell, James A. Baker Institute for Animal Health, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.** (NW527)A

NATURAL ENVIRONMENT RESEARCH COUNCIL

TERRESTRIAL AND FRESHWATER SCIENCES DIRECTORATE
INSTITUTE OF TERRESTRIAL ECOLOGY

Head of Merlewood Research Station, Cumbria

Up to £24,302

Applications are invited for the position of Head of the ITE Merlewood Research Station, at Grange-over-Sands, Cumbria. The Institute of Terrestrial Ecology is part of the Terrestrial and Freshwater Sciences Directorate and has 6 stations distributed throughout Britain. Merlewood, with a staff of about 50, undertakes strategic and applied research in terrestrial ecology with particular emphasis on quantitative forest and plant ecology, soil biology, vertebrate ecology, radionuclide pathways and land use. Research is carried out in the UK and overseas, including contract work for DOE, MAFF, ODA, CEC, NCC and others. The research at Merlewood is co-ordinated with that at other ITE and TFS Stations. The ITE Chemical Service, Library Services and a NERC Stable Isotope Facility are based at Merlewood

The Head of Station is responsible to the Director of Terrestrial Ecology (North) for the organisation, management and development of research and resources, for stimulating contract income, and for maintaining discipline and morals at Merlewood in line with the policies of ITE. The Head is also responsible for fostering co-operation with other ITE Stations, NERC Institutes and other research organisations including universities.

Applicants should have a distinguished research record in a relevant area of science and experience to management.

The appointment is pensionable under the NERC Superannuation Scheme. Terms and conditions are similar to those in the British Civil Service.

The Natural Environment Research Council is an Equal Opportunities Employer. All jobs in the UK are open to men and women of all ethnic groups and the Council also welcome applications from disabled people.

Application forms may be obtained from Mrs June Shields, Natural Environment Research Council, Polaris House, North Star Avenue, Swindon, Wilts SN2 1EU, or by telephoning (0793) 40101 ext 472.

Closing date for completed applications, including full CV is 19 March 1987. (2238)A

Natural
Environment
Research
Council

EMORY UNIVERSITY
Department of Physics

RESEARCH ASSOCIATE

position in biophysics is available on or after 1 June 1987. One year with renewal possible. The research involves Mössbauer and EPR studies on metalloproteins; will have opportunity to interact with chemistry and biochemistry research groups. Recent doctorates in physics, biophysics, or physical biochemistry are invited to apply. Experience in Mössbauer spectroscopy and in electronics is desirable.

Send curriculum vitae and names of three references to: **Dr B.H. Huynh, Physics Department, Emory University, Atlanta, GA 30322.**

Emory University is an Affirmative Action/Equal Opportunity Employer. (NW544)A

WASHINGTON UNIVERSITY
MEDICAL SCHOOL

ASSISTANT PROFESSOR

DEPARTMENT OF PHARMACOLOGY

Applications are invited from outstanding candidates for a highly competitive tenure track faculty position. Extensive postdoctoral experience emphasizing mechanistic approaches to pharmacological problems are preferred. Send curriculum vitae, reprints, a description of current/future research plans, and 3 letters of reference to: **Dr. Philip Needleman, Department of Pharmacology, Washington University Medical School, 660 S. Euclid, St. Louis, MO 63110. (AA/EOE)** (NW500)A

UNIVERSITY OF KENT AT CANTERBURY
BIOLOGICAL LABORATORY
POSTDOCTORAL AND TECHNICIAN POSTS IN ANIMAL CELL BIOTECHNOLOGY

This Laboratory has recently been selected as a major research centre in the new SERC Biotechnology Directorate initiative in Animal Cell Culture. The programme is funded in part by a consortium of five leading British biotechnology companies, and at Kent the focus will be on (1) Maintenance of hybridoma genotype and antibody production in continuous culture, and (2) Optimisation of growth and recombinant protein production in Chinese Hamster Ovary cells. Applications are invited from scientists at the senior postdoctoral, junior postdoctoral and senior technician levels to join the multidisciplinary research team working on this programme. Candidates are sought with one or more of the following areas of expertise: FERMENTATION TECHNOLOGY; CULTURE OF HYBRIDOMAS AND ANIMAL CELL LINES; IMMUNOLOGY; GENETIC MANIPULATION OF EUKARYOTIC CELLS; BIOCHEMISTRY OF MAMMALIAN CELL GROWTH.

Apply by letter with full curriculum vitae and the names and addresses of two referees to the Senior Assistant Registrar, Faculty of Natural Sciences, The Registry, The University of Kent, Canterbury, Kent CT2 7NH. Please quote reference A87/37. (2199)A

RESEARCH ASSOCIATE/ POSTDOCTORAL FELLOW

The Mass Spectrometry Facility of the University of California, San Francisco is seeking a uniquely qualified PhD **biochemist** to participate in several ongoing long-term research projects studying the carbohydrate structure of glycoproteins.

Preferred qualifications: (1) extensive experience in oligosaccharide isolation and purification, (2) some experience in the use of gas chromatography/mass spectrometry for carbohydrate structural analyses, (3) strong interest in development of advanced high mass mass spectrometry for study of intact oligomers. Salary will be commensurate with applicant's qualifications and experience.

Send curriculum vitae and 3 letters of recommendation to:

Prof. Susan J. Fisher and
Prof. A.L. Burlingame
Department of Pharmaceutical Chemistry
University of California, San Francisco
San Francisco, CA 94143-0446

University of California, San Francisco, is an Equal Employment/ Affirmative Action Employer. Women and minorities are encouraged to apply. (NW541)A

NEUROSCIENCES

The Kinsmen Laboratory (Department of Psychiatry, U.B.C.) invites applications from outstanding individuals with a commitment to excellence in research and teaching in nueroscience for a tenure-track position at the rank of assistant professor. The appointee will be expected to develop an independent research program. Applicants should have a Ph.D. and/or M.D. degree and have shown clear evidence of a capacity for productive original research. The laboratory offers excellent research facilities and collaborative, interdisciplinary research is encouraged, as is collaboration with clinical psychiatrists and neurologists. The laboratory is located in the psychiatric hospital; adjacent hospitals have research clinics in epilepsy, movement disorders and Alzheimer's disease, as well as a PET-NMR imaging center.

Applicants should send curriculum vitae, list of publications, a brief summary of research plan, and the names and addresses of three references to: Dr. E.G. McGeer, Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., Canada, V6T 1W5.

In accordance with Canada Employment and Immigration regulations, priority for this position will be given to Canadian citizens and permanent residents. (NW560)A

MOLECULAR GENETICIST-NEUROBIOLOGIST

Tenure track position in the Andrus Gerontology Center and the Department of Biological Sciences. To establish a new laboratory research program in the general area of molecular genetics-neurobiology, with strong interests in aging and age-dependent neurological disease. This laboratory will be affiliated with the new university-wide Neural, Informational and Behavioral Sciences (NIBS) program. This position is being recruited at the Assistant Professorial level. However, more senior appointments will be considered. Please send curriculum vitae, bibliography, names of four references and a brief description of career goals and research objectives by 1 May 1987 to: **Dr Caleb Finch, Chairman, Search Committee Molecular Geneticist-Neurobiologist, Department of Biological Sciences, University of Southern California, University Park, Los Angeles, California 90089-0371.**

The University of Southern California is an Equal Opportunity/ Affirmative Action Employer. (NW534)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(UNIVERSITY OF LONDON)
DEPARTMENT OF IMMUNOLOGY
POSTDOCTORAL RESEARCH FELLOW

required to work on the antigen receptor of T cell clones reactive with HLA molecules. Experience in cellular immunology and tissue culture techniques is highly desirable, and a biochemical background would be an added advantage. The post is funded until 31 December 1988 by a programme grant from the MRC to Professor J.R. Batchelor. Suitable applicants from all EEC countries are welcome.

Further particulars available from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS. Applications including a c.v. and the names of two referees should be sent to the Personnel Office, at the above address. Please quote reference A1A2/S. Closing date: 12th March 1987. (2171)A

UNIVERSITY OF GLASGO
DEPARTMENT OF DERMATOLOGY
RESEARCH ASSISTAN

Applications are invited for above position which is funded an MRC programme grant u the end of December, 1988. T work involves studies on keratin intermediate filaments a desmosomes in normal a diseased epidermis and previo experience in protein/membra biochemistry would be an adva tage.

The appointment could be ma at either the pre- or postdocto level and salary will be at appropriate point on Range 1A 1B of the scales for Research a Analogous Staff depending up age, qualifications and e perience.

Applications should be made Dr D. Skerrow, Univers Glasgow, Department of Derm tology, 56 Dumbarton Roa Glasgow G11 6NU enclosing curriculum vitae and the nam and addresses of two referees. (2165)A

MRC MAMMALIAN DEVELOPMENT UNIT

Applications are invited for a

MEDICAL RESEARCH COUNCIL SHORT-TERM (3-5 YEARS) SCIENTIFIC APPOINTMENT

to work on molecular aspects of mammalian development. Candidates should possess a PhD and should have practical experience of recombinant DNA technology, and an interest in gene expression. The position is available immediately and is funded for 3-5 years with salary in the range £9,495—£12,280 per annum and £1,297 London Allowance per annum with entry to the Medical Research Council Pensions Scheme.

Applications together with a full c.v. and the names of two professional referees should be sent to **Dr Anne McLaren, MRC Mammalian Development Unit, Wolfson House (University College London), 4 Stephenson Way, London NW1 2HE.** (2194)A

THE UNIVERSITY
OF MANCHESTER
:ROBIOLOGY GROUP

DEPARTMENT OF CELL
ID STRUCTURAL BIOLOGY

POSTDOCTORAL
:SEARCH ASSOCIATE
IN MICROBIOLOGY

ications are invited for the
e post funded by SERC and
to join a group studying
growth and morphology of
rium graminearum in batch
continuous culture. The bio-
of this fungus is used to pro-
myco-protein which has been
:eted as a food for human
umption. Studies will be made
.e inter-relationships between
ıbrane phospholipid com-
:ion, the activites of enzymes
lved in wall grwoth, and
ıal branching. Previous ex-
ence with fermenters and a
arch training in microbiology
iochemistry will be of advan-
. The post can be extended up
ı maximum of three years.
al salary range on 1A scale up
,495 p.a. (under review).
rannuation.

:C CASE STUDENTSHIP
IN MICROBIOLOGY

ılications are invited for the
ve studentship, tenable from
ober 1987, to study the effect
polymeric additives on the
phology and metabolism of
diomycetes in liquid culture.
collaborating company is B.P.
ıdidates should hold, or expec-
to obtain, a first or upper
ond class honours degree in
robiology or a related bio-
cal subject.

.pplicants for these posts
uld send a curriculum vitae and
names and addresses of two
ees as soon as possible to:
essor A.P.J. Trinci, Depart-
of Cell and Structural Bio-
y, The University, Manchester
3 9PL. (2216)A

UNIVERSITY OF SURREY

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL
RESEARCH FELLOW
MOLECULAR BIOLOGY

Suitably qualified candidates are invited to apply for this post with an active research group using recombinant DNA and gene-transfer techniques to study the role of selenium-dependent glutathione peroxidase (GPX) in human disease. The group occupies the new Molecular Biology Laboratory in the Department of Biochemistry. The research will involve the use of cloned DNA probes to study GPX gene expression/regulation in human tissue and to correlate human GPX polymorphism with susceptibility to cellular peroxidative damage in certain GPX-related diseases. The successful candidate will be expected to interact closely with other members of the research group and with clinical colleagues. Relevant experience in biochemistry, molecular biology or molecular medical genetics would be an advantage although training could be given to an outstanding candidate wishing to enter the field.

The post, which is funded by the Wellcome Trust for 3 years, is available from April 1987. Starting salary will be in the range £8,020—£9,495 (under review) on the RA1A scale, depending on age and experience. Further information can be obtained from Dr P. Goldfarb on 0483-571281 ext. 2534.

Applications (3 copies) including a full curriculum vitae and the names of 2 referees should be sent to the Personnel Office (JLG), University of Surrey, Guildford, Surrey, GU2 5XH, quoting reference no: 600. Closing date: Tuesday, 10th March 1987. (2190)A

DIRECTOR
Molecular Biology

Henry Ford Hospital is currently seeking leadership for a new group to be established in molecular biology. The group would consist of 4 investigators, with the specific research fields not rigidly defined. Some possible areas are lymphocyte activation and differentiation, gene expression, oncogenes, lymphokines, growth factors, transplantation biology, immunology, and DNA probes. Our goal is to establish a high quality fundamental research program. The director would recruit 3 colleagues and provide overall direction for the program. Substantial support and space will be provided, and potential academic affiliations are feasible. The person we seek should be an acknowledged leading scientist, with a substantial publication record and history of peer-reviewed grant support.

Interested applicants should forward a c.v. and the names of 3 references to: **Dr Joseph D. Shore, Director of Research, Henry Ford Hospital, 2799 W. Grand Blvd., Detroit, MI, 48202.**

(NW539)A

LUDWIG INSTITUTE FOR CANCER RESEARCH
MONTREAL BRANCH

CELL BIOLOGIST

The Ludwig Institute has been newly established in Montreal to conduct basic and clinical research in conjunction with the Royal Victoria Hospital and the McGill University Faculty of Medicine. Our program is directed toward understanding the relationship between normal organismal development and embryonal tumors.

We now seek an individual with research interests in cell-cell and cell-substratum interactions, the formation of specific tissue architecture and aberrancies in these processes related to neoplasia to head our Laboratory of Cell Biology. Applicants should have postdoctoral experience, a record of significant accomplishment in this area and an interest in appropriate collaborative interactions with our Laboratories of Molecular Genetics, Developmental Biology, Clinical Research and Molecular Oncology.

The appointment will be at LICR levels equivalent to assistant or associate professorship; corresponding academic appointments will be possible through McGill University. Support for salary, relocation, laboratory personnel, equipment and supplies will be provided. Preference will be given to qualified Canadian citizens or permanent residents.

Written applications including a curriculum vitae, names of three referees and a short synopsis of research accomplishments and directions should be received no later than May 1, 1987 by: W.K. Cavenee, Director, Montreal Branch, Ludwig Institute for Cancer Research, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1. (NW559)A

LABORATORY DIRECTOR
IN VITRO FERTILIZATION

Ph.D. to direct human I.V.F. and reproductive endocrinology in a full-time clinical laboratory, research, and academic faculty position in the Department of Obstetrics and Gynecology, George Washington University Medical Center, Washington, D.C. Expertise in skills needed for human in vitro fertilization is required, including gamete and embryo biology and tissue culture techniques. Experience in human I.V.F. is especially useful, with R.I.A., monoclonal antibody, cryopreservation, and andrology expertise desirable. Salary and rank dependent on experience.
Send curriculum vitae to: **Robert J. Stillman, M.D., Director, Division of Reproductive Endocrinology and Fertility, Department of OB/GYN, George Washington Medical Center, 2150 Pennsylvania Avenue, NW, Washington, D.C. 20037.** *George Washington University is an Equal Opportunity/Affirmative Action Employer.* (NW557)A

UNIVERSITY OF NEWCASTLE UPON TYNE
in collaboration with
CASTROL LIMITED

Following several years of effective collaborative research, Castrol are intending to support a major research project at the University of Newcastle concerned with the metabolic fate of their products and their impact on the environment. Applications are invited for a Research Associateship (minimum salary £10,375, under review) from graduates who have already had 2-3 years of post-doctoral experience. A background in microbiology or biochemistry would be most pertinent but applicants with experience in other experimental biological or environmental sciences will be considered. The appointee would not only make his/her own unique contributions to the project but would also have day-to-day supervision for the related research of several CASE students and graduate assistants whose studies are either underway or will commence within the tenure of the Associateship. The post will be for a period of 3 years.

Applications, including a detailed curriculum vitae and the names of three referees should be directed to **Professor R.B. Cain, Department of Agricultural Biology, The University, Newcastle upon Tyne, NE1 7RU from whom further details may be obtained. Short-listed applicants will be interviewed both at Newcastle and at Pangbourne.**

The closing date for applications is 20th March 1987. (2234)A

LABORATOIRE DE GENETIQUE MOLECULAIRE DES EUKARYOTES

Molecular Immunology
Autoimmune Disease Models

POSTDOCTORAL POSITION

A postdoctoral position is available to study the molecular biology of autoimmune disease, in particular, the role of major histocompatibility complex proteins in the development of diseases such as multiple sclerosis and diabetes. Approaches involve the use of transgenic mouse models, viral vector targetting, and protein and promoter engineering. The lab focusses on the molecular genetics of the MHC and is part of an institute of molecular biology whose strength is the study of eukaryotic gene regulation.

Experience in either molecular biology, cellular immunology or virology is preferred. Knowledge of French is not required. Please send a C.V. and letters of recommendation to: **Dr. D. Mathis et C. Benoist, Laboratoire de Génétique, Moléculaire des Eukaryotes, Faculté de Médecine, 11, rue Humann, 67085, Strasbourg Cédex.**

(W3841)A

LANCASHIRE POLYTECHNIC AT PRESTON
School of Applied Biology
RESEARCH ASSISTANT

Applications are invited for a Wellcome Trust supported Research Assistantship, available immediately, to investigate stimulus-secretion coupling in the exocrine pancreas.

A good honours degree in Physiology, Biochemistry or Pharmacology is desirable. Registration for a higher degree may be possible.

Salary in the range: £7,371 to £8,196.

Application form and further details quoting reference: AA/437, obtainable from the **Personnel Office, Lancashire Polytechnic, Preston, PR1 2TQ. Tel: (0772) 262027.**

Closing date: 27 March 1987. (2243)A

RESEARCH ASSISTANT (NON-CLINICAL)

required to conduct experiments on the neurophysiology of single-cells in the vertebrate retina. Candidates should have a postgraduate degree and relevant practical experience. Appointment for three years. Commencing salary £9,898 p.a. (including London Allowance).

Applications to Professor G.B. Arden, Institute of Ophthalmology, Judd Street, London, WC1H 9QS, giving details of qualifications and experience, and day-time telephone number.

(2169)A

INSTITUTE OF HORTICULTURAL RESEARCH
East Malling
PLANT HORMONE RECEPTORS

A Higher Scientific Officer is required for 34 months to join a research programme involving collaboration with four other EEC Laboratories. Receptors for auxin, auxin transport inhibitors and fusicoccin will be studied, and attempts to isolate a native auxin transport inhibitor (phytotropin) will form a major emphasis.

Qualifications:

First or Upper Second honours degree in biological sciences, with at least 2 years relevant postgraduate research, preferably to PhD level. Experience of natural products isolation and/or immunological methods useful.

Salary in range £8,405 - £11,323 according to qualifications and experience. Non-contributory superannuation scheme. Further details and application form from Personnel Officer (ref 2/EM), Institute of Horticultural Research, Bradbourne House, East Malling, Maidstone, Kent ME19 6BJ by 6 March 1987.

The AFRC is an Equal Opportunities Employer. (2223)A

UNIVERSITY OF EXETER
DEPARTMENT OF GEOLOGY

Applications are invited for a

Temporary Lectureship in GEOCHEMISTRY

tenable from 1 October 1987 for one year. Teaching duties will cover igneous and metamorphic petrology.

Salary will be within the range £8,020 — £9,495 pa (under review) with placement dependent on age and experience.

Telephone the Personnel Office (Exeter 263100) for further particulars, quoting reference no 3502. Closing date 18 March 1987.

(2241)A

THE INSTITUTE OF CANCER RESEARCH

(UNIVERSITY OF LONDON)

POSTDOCTORAL RESEARCH SCIENTIST: DRUG DEVELOPMENT

Applications are invited for the above vacancy within the Drug Development Section at the Institute's laboratories in Sutton, Surrey, from persons having experience of *in vivo* and *in vitro* drug metabolism and pharmacokinetic studies using experimental animals, preferably with experience with radio-labelled substrates. A working knowledge of HPLC would be an advantage. The successful candidate will join a multi-disciplinary team concerned with the synthesis and development towards clinical trials of novel chemotherapeutic agents for the treatment of steroid hormone-dependent breast and prostatic cancer.

The appointment is for three years. Salary in the range of £8,020 — £11,790 p.a. plus London Allowance of £1,297.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications, including a full curriculum vitae and the names and addresses of at least two referees should be sent in duplicate to The Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting Ref. No: 2/87/S/NA/59. (2180)A

HIGHER SCIENTIFIC OFFICER
ORNITHOLOGIST
REFERENCE NO: HE2 36 75

Period appointment, initially for 3 years, with a possibility of extension up to 5 years. The post will be based at Peterborough.

NCC has a responsibility to advise government as to the fulfilling of its international commitments to conservation, including those under the EC Director on the conservation of wild birds. An ornithologist is required to collate and review existing data on the extent to which site-safeguard meets these commitments, and to assist on the development of conservation measures for wide-ranging species.

Qualifications: A degree or equivalent in a biological subject plus at least 2 years relevant post-graduate research experience. Wide ornithological knowledge, writing experience and the ability to collate diverse data are essential. Computing and statistical expertise and a knowledge of land-use practices would be advantageous.

Salary: Between £8,405 and £11,323 dependent upon qualifications and experience. The appointment is pensionable.

Application forms and further details are available from: Personnel 1, The Nature Conservancy Council, Northminster House, Peterborough PE1 1UA. The closing date for completed applications is 19 March 1987. Interviews will be held in late April.

NCC is an equal opportunities employer. (2233)A

THE AUSTRALIAN
NATIONAL UNIVERSIT

Applications are invited from s
ably qualified women and men
appointment to the follow
position:

RESEARCH SCHOOL
OF BIOLOGICAL SCIENC
NEUROBIOLOGY
(VISUAL SCIENCES GROUP)
POSTDOCTORAL FE
GRADE I

The appointee would be expec
to participate in the group work
under Professor Horridge and
Srinivasan in visual processing
arthropods, especially insects.

Applicants should hold,
expect to have completed a P
degree during the first half of 19
Knowledge of standard neu
physiology techniques is essent
preferably with experience in d
marking of neurons, on-line d
processing and microelectro
methods. As an alternati
appointment of a specialist
honeybee visual discrimin
tasks would also be conside
Closing date: 31 March 1987.
BS.5.2.1.

Conditions of appointme
Salary will be in accordance w
qualifications and experie
within the range: Postdocto
Fellow Grade 1 (at a fixed poi
$A24,013—$27,507 p.a.; appoi
ment will be: normally two yea
with the possibility of extension
maximum of three years. Gra
are provided towards travel a
removal. Eligible appointees
be required to join the Super
nuation Scheme for Austral
Universities. Maternity leave
available. The University reser
the right not to make an appo
ment or to make an appoint
by invitation at any time.

Applicants should quote
advertisement reference w
requesting further particulars
when submitting applicati
which should be forwarded to
Registrar, Australian Natio
University, G.P.O. Box 4, C
berra, ACT 2601, Austra
Further particulars also availa
from the Association of Comm
wealth Universities (Appts),
Gordon Square, London WC
0PF. The University is an eq
opportunity employer.

(W3848)

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nature

the widest
international
selection of jobs
in science
— EVERY WEEK

ERSITY
ONG KONG
URER IN BOTANY

lications are invited for a
ureship in the Department of
iny. Candidates should have
nterest in Plant Ecology or
e other aspects of environ-
tal Botany. The appointee
t be prepared to teach intro-
ory courses in Botany and/or
it Systematics.

nual Salary (superannuable)
on an 11-point scale:
$176,880—295,680 (approx.
860—24,850; sterling equiva-
at February 6, 1987). Starting
ry will depend on qualifica-
s and experience. At current
s, salaries tax will not exceed
of gross income. Housing
efits at a rental of 7½% of
ry, children's education allow-
es, leave and medical benefits
provided.

urther particulars and applica-
forms may be obtained from
Secretary General, Associa-
of Commonwealth Univer-
s (Appts), 36 Gordon Square,
don WC1H 0PF, or from the
pointments Unit, Registry,
iversity of Hong Kong, Hong
ng. Closes: 4 April 1987.

(W3850)A

MEDICAL RESEARCH COUNCIL
MRC CLINICAL AND POPULATION CYTOGENETICS UNIT
NON CLINICAL SCIENTIST II

plications are invited for a short
n non-clinical scientific post
e molecular developmental
ology group at the above Unit.
e position, funded in the first
stance for one year is to join
ojects already in progress which
m to elucidate the role of the
meo-box genes in regulating
ouse embryonic development.
ndidates of immediately post-
ctoral or equivalent status will
preferred. Experience in one
all of the following techniques
ll be an advantage: cell culture,
combinant DNA, immunology
d protein chemistry. However,
terested candidates lacking such
perience will be considered, and
-service training will be provided
ithin this expanding group.

Renumeration will be at an
opriate point on the scales
r university non-clinical
ademic staff. Further details
om, and applications with names
d addresses of two referees, to
e Administrator, MRC Clinical
d Population Cytogenetics
nit, Western General Hospital,
rewe Road, Edinburgh EH4
XU, by 6 March 1987, quoting
eference IJ/2/87.

An Equal Opportunity
mployer. (2120)A

LEEDS WESTERN HEALTH AUTHORITY
Clarendon Wing
RESEARCH ASSISTANT

Salary on the Basic Grade Biochemist scale £7,452—£9,360.

You are required to work on the molecular biology of abnormalities in sexual differentiation in mammals, including man in the Department of Chemical Pathology and Department of Obstetrics & Gynaecology. The appointment will be made for a fixed period of three years from 1 April 1987.

Candidates should possess an honours degree or recognised equivalent in Biochemistry, genetics or other appropriate biological science. You may be able to register for a higher degree.

Informal enquiries to Dr A.D. Stewart (0532) 432799, ext. 3927 are invited.

Application forms and further particulars may be obtained from the Personnel Officer, Clarendon Wing, Belmont Grove, Leeds LS2 9NS. Tel: (0532) 432799, ext. 3205. Closing date 19th March, 1987. (2193)A

UNIVERSITY OF CAMBRIDGE
Department of Pathology
Division of Virology
POSTDOCTORAL RESEARCH ASSISTANT

required to join a group working on the molecular biology of influenza virus, and in particular on mechanisms of virus RNA synthesis. The project will involve analysis of the structure and function of the virus RNA-dependent RNA polymerase through the use of an *in vitro* expression system which allows the production of individual polypeptide components of the enzyme from cloned DNA, together with a comprehensive range of specific antisera directed against defined regions of the polymerase-associated polypeptides.

The position is funded by the Medical Research Council for a period of 3 years and is available in an active Department with a wide range of interests in molecular virology. Experience in molecular biology and/or protein biochemistry would be an advantage, but a background in virology, while helpful is not essential. Salary according to age and experience.

Applications in writing with curriculum vitae and the names and addresses of two referees should be sent to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. Please quote 9533. Further details from Dr S C Inglis. Tel: (0223) 336917.

(2205)A

DIRECTOR OF THE LABORATORY OF CLINICAL MICROBIOLOGY

The Wadsworth Center for Laboratories and Research is seeking a scientist-administrator to assume a leadership role in research and public health-related responsibilities in the area of clinical microbiology. The professional staff in these laboratories encompasses desciplines of bacteriology, mycology, parasitology, diagnostic immunology, virology; mycobacteriology, and related areas. We are seeking an individual with in-depth experience and interest in clinical areas related to these disciplines together with an interest in epidemiology. This is a senior level position with significant administrative responsibilities and expectation for leadership in program development related to public health problems and policy. Specific research activities are expected and encouraged. Applicants must have a PhD or MD with relevant clinical laboratory and research experience with proven ability to assume administrative responsibilities.

Please send curriculum vitae and the names of three references to: Felix H. Heilpern, Wadsworth Center for Laboratories and Research, Empire State Plaza, Albany, NY 12201. *An Affirmative Action/Equal Opportunity Employer.* (NW542)A

DEPARTMENT OF PHYSICS AT YARMOUK UNIVERSITY

invites applications for faculty positions available in the following fields: Astronomy, condensed matter physics and plasma physics.

Candidates must possess a PhD degree in any of the above mentioned fields.

Applications including copies of academic and professional transcripts and recommendation letters should be sent not later than April 15, 1987 to: Academic Staff Affairs Division, Yarmouk University, Irbid, Jordan.

(W3846)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)
Department of Clinical Oncology
POSTDOCTORAL FELLOW

Required to join an exciting new research group involved in the application of molecular biology to the treatment of cancer. The successful applicant will be involved in the construction of human monoclonal antibodies to tumour related proteins. Experience in recombinant DNA work essential. The department's laboratories are now open and provide an attractive environment for interfacing basic science with a dynamic clinical group. Further details can be obtained from Professor Karol Sikora on 01 740 3060.

The position is supported by the CRC for three years.

Salary depending on qualifications and experience, up to £9,390 (under review) plus £1,393 London Allowance.

Application forms and further information may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0HS quoting Ref: ARSI/F. Closing date: 12 March 1987. (2220)A

UNIVERSITY OF BRISTOL
Department of Biochemistry
GRADUATE RESEARCH ASSISTANT

Applications are invited for a Research Assistant to work on the long-term regulation of the enzyme glutaminase in liver The work will involve cell culture and immunological techniques. The post is funded by the Medical Research Council and is available for two years.

Applicants should possess a first degree in Biochemistry or a closely related subject. Experience of cell culture would be an advantage, but is not essential Salary is on the Range 1B (£7,055 - £9,495) Applications including a cv and the names of two referees should be sent as soon as possible to Dr J D McGivan, Department of Biochemistry, Medical School, University Walk, Bristol BS8 1TD (0272 303030 ext 4965) from whom further details can be obtained

(2207)A

IMPERIAL COLLEGE
(University of London)
POSTDOCTORAL RESEARCH ASSISTANT

Required for a 3-year NERC funded post to carry out experimental and theoretical studies in plant herbivore dynamics in collaboration with Dr M J Crawley at Silwood Park, Berkshire. The study will involve manipulation of plant and insect densities under field conditions with the aim of developing more realistic models of population dynamics. Experience in quantitative ecology advantageous.

Salary £8,020 - £9,495 (under review) - depending upon age and experience. Starting date negotiable.

Further particulars from and applications with CV, covering letters and names of 2 referees to Dr M J Crawley, Imperial College at Silwood Park, Ascot, Berks. SL5 7PY. Tel: 0990 23911.

(2211)A

ASSISTANT PROFESSOR AND ASSISTANT DIRECTOR CYTOGENETICS LABORATORY

DEPARTMENT OF LABORATORY MEDICINE & PATHOLOGY UNIVERSITY OF MINNESOTA

A tenure track faculty position is available for candidates who hold either an M.D. degree with subspecialty training in Human Genetics or a Ph.D. degree in Human Genetics or closely-related basic science. Candidates must have a minimum of one year of experience in a clinical cytogenetics laboratory, and be Board eligible or certified as a Clinical Cytogeneticist by the American Board of Medical Genetics. Demonstration of research productivity is necessary.

The person in this position will be involved in teaching cytogenetics to physicians in practice, medical students, graduate students, postgraduate residents, postdoctoral fellows, allied health students, and technologists. The individual will be expected to develop an independent, basic or clinical research program in an area of interest to him/her.

The successful candidate will serve as Assistant Director of the Cytogenetics Laboratory, and will assume partial responsibility for the daily operation of this laboratory. Specifically, this individual will be responsible for the supervision and training of technologists analyzing peripheral blood specimens for constitutional abnormalities, and for interpreting the results of these analyses. Expertise in current methodology and development of new techniques for cytogenetic analysis is expected.

Applicants should send curriculum vitae and three letters of recommendation to: **Harry Orr, Ph.D., Box 198 UMHC, Dept. of Laboratory Medicine & Pathology, 420 Delaware Street SE, Minneapolis, MN 55455**

Deadline for receipt of applications is March 31 1987. The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities. (NW537)A

AWARDS

NUFFIELD FOUNDATION

Awards to Newly Appointed Science Lecturers in Science, Applied Science and Engineering

The Trustees of the Nuffield Foundation are offering a limited number of awards of up to £4,000 to newly appointed University and Polytechnic lecturers in Science, Applied Science and Engineering.

The object of the awards is to give assistance to such lecturers in the early stages of their independent research careers.

Awards will be made competitively and preference will be given to lecturers wishing to explore novel and original lines of research that may not yet be at a stage suitable for Research Council support. Fifty four awards were made in 1986.

ELIGIBILITY

Applicants must be newly appointed to lectureships or equivalent posts in Science, Applied Science, Mathematics, Engineering or Medical Departments. Candidates must apply within 12 months of taking up their posts and may apply between the time of being appointed and taking up the post.

HOW TO APPLY

There are two selection meetings a year. The next closing date for applications is 31 March 1987. Applications must be on the appropriate form, which is available from the Nuffield Foundation, 28 Bedford Square, London WC1B 3EG.

The scheme is only open to applicants from institutions within the United Kingdom

(2175)N

GRANTS & SCHOLARSHI

THE NUFFIELD FOUNDATION OLIVER BIRD FUND FOR RESEARCH INTO RHEUMATISM

Applications are invited for research grants for work directed to the better understanding, and treatment, of rheumatic disease. The Oliver Bird Fund, from which over £200,000 was spent on research grants during 1986, is administered by the Trustees of the Nuffield Foundation on the advice of an expert committee whose chairman is Dr D A J Tyrrell, CBE, FRS. Grants may be made only for research in UK institutions.

Further details of how to apply are available from the Nuffield Foundation. The closing date for applications is 31 March 1987.

Applications will be considered at a meeting in May 1987.

Nuffield Foundation
28 Bedford Square
London WC1B 3EG

(2174)H

ASSISTANTSHIPS

DURHAM UNIVERSITY

DEPARTMENT OF PHYSICS

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANTSHIP

tenable for two years. The successful applicant will participate in observational and theoretical studies relating to the properties of Giant Molecular Clouds in this and other galaxies.

Initial salary up to £9,000 p.a (under review) with superannua tion. The starting date is 1s October 1987.

Applications (3 copies), namin three referees, quoting ref. P should be sent by 14th March 198 to the Registrar, Science Labor tories, South Road, Durhan DH1 3LE, from whom furth particulars may be obtained. (2184)

STUDENTSHIPS

RESEARCH STUDENTSHIP

Applications are invited for the newly established **Dee Studentship.** This award is restricted to students from the U.K. or European Community who wish to be candidates at the University of Cambridge for a mathematics degree in Natural Sciences, Mathematics, Computing Sciences, Management or Economics. The award covers full fees and maintenance at slightly above the U.K. Research Council grant level and is available from October 1987.

Details and application forms may be obtained from the Tutor for Advanced Students, Churchill College, Cambridge CB3 0DS. The closing date for applications will be 15th May 1987. (2208)F

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENTS OF CLINICAL GENETICS AND ENDOCRINOLOGY

PhD STUDENTSHIP

Applications invited for a three year research studentship leading to a PhD. The project involves structural analyses of the regulatory regions of the human insu gene.

Applicants should have expect to have a good honou degree in one of the biologic sciences. Applications shou include a curriculum vitae a names of two referees and shou be sent to Dr M.W. Kilpatric Department of Clinical Geneti University of Birmingham, B mingham Maternity Hospit Birmingham B15 2TG. Telepho 021 472 1301 ext. 2602. (2167)F

STUDENTSHIPS continued

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
WOLFSON MOLECULAR BIOLOGY UNIT

Ph.D. STUDENTSHIPS

Applications are invited from candidates who have or expect to have a first or upper second class honours degree in biochemistry, molecular or cell biology or a related subject.

Successful applicants will join a team of research scientists working in well-equipped laboratories using molecular biological techniques to investigate parasitic protozoan and viral infections. Areas of research include analysis of cloned surface antigen molecules, gene expression, cell biology and diagnostics.

Please send curriculum vitae and the names and addresses of three academic referees to: The Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT. (2232)F

UNIVERSITY OF LIVERPOOL

Department of Physiology

RESEARCH STUDENTSHIPS

Applications are invited for studentships tenable for three years from students who have or expect to obtain a first or upper second class honours degree. The following projects will be offered:

Professor O H Petersen — Control of potassium ion channels in insulin-secreting cells. Professor G J Dockray — Phosphorylation of gut hormones. Dr R Dimaline — Physiology and biochemistry of VIP-like peptides. Dr R Burgoyne — The role of the cytoskeleton in the control of axonal growth. Mr D V Gallacher — Neurotransmitter regulation of electrolyte and water fluxes across transporting epithelia. Dr R J Gayton and Dr D Taylor — Physiology and pharmacology of hypothalamic neurons receiving input from cutaneous and visceral receptors. Dr H J Tracy — A study in anesthetised ferrets of the neurohormonal mechanisms involved in the release of antral gastrin by distension.

Further details may be obtained from Dr R D Burgoyne (Tel: 051 709 6022 ext 2446). (2125)F

POSTGRADUATE STUDENTSHIP
in the
LEUKAEMIA RESEARCH FUND CENTRE
at the
INSTITUTE OF CANCER RESEARCH

A postgraduate studentship is available to study molecular mechanisms of leukaemogenesis. The successful candidate would join a team currently cloning the chromosomal translocation breakpoint from the DNA of human leukaemia cells carrying the Philadelphia chromosome in order to examine the mechanisms of proto-oncogene activation.

The cloning and characterization of transcription products of the translocated and normal genes will be necessary, leading to transfection and/or transgenic experiments designed to analyze the leukaemogenic potential of these sequences and assay for dominant effects of the altered gene products.

Applicants are advised that in the majority of the Institute's premises smoking is prohibited.

Applications, in duplicate, including the names and addresses of two referees should be sent to the Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL. (2179)F

UNIVERSITY OF OXFORD
RESEARCH STUDENTSHIPS

Applications are invited from students interested in postgraduate study in the Department of Anatomy where opportunities for research in developmental biology, neurobioloy, endocrinology, or membrane physiology are now available. Radioimmunoassay, autoradiography, tissue culture electron and scanning electron microscopy, quantitative image analysis and biochemical equipment are among the facilities offered by the Department. Details of research in the Department can be obtained by applicants.

Applicants, who should have or expect to obtain a good honours degree, should send a copy of their curriculum vitae, a statement of their research interests, and the names of three referees to **Professor R W Guillery FRS, Department of Human Anatomy, South Parks Road, Oxford, OX1 3QX,** by 1 May 1987 at the latest. (2229)F

MRC BRAIN METABOLISM UNIT
University Department of Pharmacology,
1 George Square, Edinburgh EH8 9JZ
RESEARCH STUDENTSHIPS

Applications are invited from final year students expecting to graduate with a first or upper second class honours degree in appropriate branches of science, medicine or veterinary medicine. The successful applicants will work on one of the following topics: molecular and cellular mechanisms in the regulation of pituitary hormone secretion; steroid influences on gene expression; control of tachykinin gene transcription; role of serotonin in corticotrophin secretion; neuropharmacology of cerebral blood flow and metabolism; immunological studies of Alzheimer's Type Dementia and Down's syndrome. The applicants will be registered as research students in the University of Edinburgh.

Applications including a full curriculum vitae together with the names of two professional referees should be addressed to the Administrator, Mrs Anne Milligan, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ no later than April 6, 1987 (2176)F

FELLOWSHIPS

UNIVERSITY OF EDINBURGH
Chemistry Department
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for this post, to work on the determination of molecular structures in the gas phase by electron diffraction. The work may also include analysis of data obtained by rotational, vibrational and liquid crystal nmr spectroscopy.

The appointment will be for two years, with the possibility of an extension, beginning October 1987 or earlier. Salary will be on the Research IA Scale, currently £9,000 - £12,780 pa (under review).

Applications, with full curriculum vitae and names and addresses of two referees should be sent to Dr D W H Rankin, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ, Scotland, as soon as possible. Please quote Ref No: 5445. (2210)E

POSTDOCTORAL RESEARCH FELLOWSHIP

A postdoctoral position is available immediately to study a steroid metabolizing enzyme in liver. The applicant should be a U.S. citizen or have immegrant status, hold a Ph.D. degree (or equivalent), and have background knowledge and experience in enzymology, protein isolation, molecular biology, and cell culture.

Please send curriculum vitae and the names and addresses of two academic references to: **Dr. Leon Milewich, The University of Texas Health Sciences Center, Cecil H. and Ida Green Center for reproductive biology sciences, 5323 Harry Hines Boulevard, Dallas, Texas 75235–9051.**

The University of Texas is an equal opportunity/affirmative action employer. (NW550)E

continued on pages 22 & 23

NERC INSTITUTE OF VIROLOGY, OXFORD

Postgraduate & Postdoctoral Fellowships in AIDS Vaccine Development

Applications are invited for postgraduate and postdoctoral fellowships at the Institute of Virology in Oxford concerning vaccine development for AIDS. The 3 year appointments are to undertake research into the expression of selected genes representing human immunodeficiency viruses in order to develop candidate subunit vaccines.

One appointment is at Senior Scientific Officer level the other at Scientific Officer level.

Applicants for the SSO post should have a PhD or equivalent degree in an appropriate scientific discipline with at least 4 years postgraduate experience. Applicants for the SO post should have interest or experience in recombinant DNA technologies.

Salary will be in the range of £6,711 to £9,225 for the SO appointment and £10,558 to £13,762 for the SSO. Starting salary may be above minimum, according to age and qualifications. Staff of the council are not Civil Servants, but their pay and conditions are similar to those of the Civil Service.

The Natural Environment Research Council is an Equal Opportunities Employer. All jobs in the UK are open to men and women of all ethnic groups and the Council also welcomes applications from disabled people.

Further information is available from the Director, Professor D.H.L. Bishop, Institute of Virology, Mansfield Road, Oxford OX1 3SR. Telephone Oxford 512361. Closing date for applications is 13 March 1987. (2212)E

Natural
Environment
Research
Council

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(UNIVERSITY OF LONDON)

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

JUNIOR RESEARCH FELLOWSHIP

Applications are invited from graduates or students with a good honours degree in Biochemistry or related subjects for a three year Junior Research Fellowship. The successful candidate will register for a higher degree in the University of London. The project concerns the molecular biology of the regulation of calcium in normal and neoplastic endocrine cells. The conditions of employment and salary are similar to the SERC/MRC studentships.

A curriculum vitae and the names of two academic referees should be sent as soon as possible to Professor B.A. Cooke, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF. Telephone 01-794-0500 extension 4202. (2217)E

GRADUATE FELLOWSHIPS IN MOLECULAR MICROBIOLOGY

The Department of Microbiology at the University of Vermont invites applications from persons interested in a career in molecular microbiology. Biochemical, immunological and molecular genetic techniques are being used to study: cytoplasmic factors affecting maintenance and control of the carcinogenic state; sexual development in higher fungi (Basidiomycetes) including isolation and function analysis of genes controlling self-incompatibility and sexual development; determinants of bacterial virulence; mechanisms of bacterial adhesion to host tissue and biological implants; response of eukaryotic cells to toxins, hormones and viruses; regulation of protein synthesis in eukaryotes; studies on increasing bioavailability of fiber by manipulating adhesion of ruminal bacteria. Full tuition remission plus a stipend of approximately $8400 per year. Write: **Dr. Thomas J. Moehring, Department of Microbiology, The University of Vermont, Given Building, Burlington, VT05405.** (NW551)E

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
Division of Parasitology
POSTDOCTORAL FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship to work with Dr D J McLaren on a WHO-funded project that addresses vaccine-induced immunity to *Schistosoma Mansoni*. The main objectives will be to analyse post skin challenge attrition, with particular emphasis on identification of parasite target antigens and host effector mechanisms. Experience in cellular and immunological techniques would be desirable. The fellowship is for a period of up to three years and is available immediately.

The salary will be in the range £9,495 to £11,790 plus £1,393 London allowance (pay award pending), with superannuation provision.

Applications should include a curriculum vitae and the names of two referees and should be sent to **Mr C R Russell, Head of Administration, NIMR, The Ridgeway, Mill Hill, London NW7 1AA by Friday 27th March 1987 quoting reference PA/871**

An equal opportunities employer. (2231)E

UNIVERSITY OF SUSSEX

SCHOOL OF CHEMISTRY AND MOLECULAR SCIENCES

POSTDOCTORAL FELLOWSHIP

Applications are invited from Organic Chemists with some experience of biological organic chemistry or Biochemists for a Postdoctoral Fellowship to work on a problem associated with the use of enzymes in synthesis, supported by the Science and Engineering Research Council.

The appointment, which is for one year in the first instance, is available from 1st October 1987 at a starting salary of £8,020 to £12,780 per annum (under review).

Applications with the names of two referees should be sent as soon as possible to Dr D.W. Young, School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QJ. (2173)E

Short-term Genetic Sequence Computer Analysis Training Fellowships —

Available at the Dana Farber Cancer Institute and Harvard School of Public Health. Housing and domestic travel support provided for 1–3 weeks. Positions open to graduate students, postdoctoral fellows, and senior researchers in molecular biology. Positions provide available software introductory training and/or collaborate research genetic sequence problem support.

For further information contact Susan Tolman, J816, Molecular Biology Computer Research Resource, **DANA-FARBER CANCER INSTITUTE,** 44 Binney St., Boston, MA 02115. *An Affirmative Action/Equal Opportunity Employer.* (NW555)E

ASTON UNIVERSITY

Department of Pharmaceutical Sciences

IMMUNOLOGIST/ BIOCHEMIST/ MICROBIOLOGIST

(Research Fellowship)

Microbiology Research Group

The Group is studying the surfac
properties of micro-organisms *i*
situ in various human infection
and is well supported from variou
outside bodies. Information i
obtained both from organism
obtained directly and without su
culture from infections and alsc
studying the antibody response t
the various surface structures c
infecting micro-organisms. Th
influence of antibiotics on the ho
organism interaction is also bein
examined.

The appointee will work on
project studying the interactio
between host and bacteria in burn
and other traumas. Of particula
interest will be the antibod
response to specific bacterial su
face components. This will b
conducted in collaboration wit
clinicians at several local hospital

The appointment will be for
period of one year in the fir
instance with the possibility c
renewal for a further period. T
commencing salary will be wi
the range £8,020 to £12,780 p
annum (presently under review).

Application forms and furth
particulars may be obtained fro
the Personnel Officer (Academ
Staff), quoting Ref: 877/6, Ast
University, Aston Triangl
Birmingham B4 7ET. Tel: 0
359 0870 (24 hour answerphone
Closing date for the receipt
applications is: 20 March 1987.
(2206)E

NATIONAL CANCER INSTITUTE

National Institutes of Health

CANCER PREVENTION FELLOWSHIP PROGRAM (CPFP)

The Division of Cancer Prevention and Control is accepting fellowship applications for a proposed August 31, 1987, entry into CPFP. Funding permitting, up to ten individuals may be accepted for three years of training. Requirments are: M.D., D.O., or an accredited doctoral degree in a discipline related to cancer prevention and research (medicine, biomedical or behavioral research, nutritional science) or equivalent; academic-professional excellence supported by official transcripts; four letters of reference; and U.S. citizenship or a resident alien eligible for citizenship within four years at the time of application. *Current salary ranges for an M.D./D.O. or a Ph.D., respectively, are $26,000 — $35,000 or $18,000 — $31,000 per annum.* Send a postcard with your name and address to **Ms. Nancy E. Garner, NIH/NCI/DCPC/CCAB, Blair Building, 9000 Rockville Pike, Room 4A01, Bethesda, MD 20892–4200,** or call (301) 427-8788 for application and information packet.

DEADLINE FOR RECEIPT OF APPLICATIONS IS MARCH 16, 1987.

An Equal Opportunity Employer.

(NW507)E

NERC INSTITUTE OF VIROLOGY, OXFORD

Postdoctoral & Postgraduate Fellowships in Virology & Genetic Engineering

Applications are invited for one postgraduate and four postdoctoral fellowships. Four of the positions concern studies of the molecular biology of arthropod-borne viruses. One postdoctoral position is to undertake research into the genetic engineering of baculovirus insecticides.

One vacancy is a three year appointment at the Scientific Officer level. Three of the vacancies are 3 year appointments at Higher Scientific Officer level. One 4 year appointment is available at Higher Scientific Officer/Senior Scientific Officer level.

For the SO position, applicants should have a degree in Biochemistry, for the postdoctoral HSO and SSO positions, applicants should have a PhD degree or equivalent in an appropriate scientific discipline with at least 2 years relevant postgraduate experience for the HSO positions and four years for the SSO post.

Salary will be in the range of £6,711 to £9,225 for the SO appointment, £8,405 to £11,323 for the HSO appointments and £10,558 to £13,762 for the SSO. Starting salary may be above minimum, according to age and qualifications. Staff of the council are not Civil Servants, but their pay and conditions are similar to those of the Civil Service.

The Natural Environment Research Council is an Equal Opportunities Employer. All jobs in the UK are open to men and women of all ethnic groups and the Council also welcomes applications from disabled people.

Further information is available from the Director, Professor D.H.L. Bishop, Institute of Virology, Mansfield Road, Oxford OX1 3SR. Telephone Oxford 512361. Closing date for applications is 13 March 1987.

(2213)E

Natural
Environment
Research
Council

NIVERSITY OF ASTLE UPON TYNE

DEPARTMENT OF IOLOGICAL SCIENCES

NIOR RESEARCH FELLOWSHIP

pplications are invited for a unior Research Fellowship to ork with Dr A.M. Derrington on e visual mechanisms of motion-etection. The appointment, hich is financed by the University, to foster research projects of utstanding potential, is for two ears, commencing as soon as may e convenient.

Candidates should preferably ave some background in sensory ysiology, perception, or comuting, and a first degree in a lated subject. Candidates execting to graduate this year will considered. Salary will be on Range 1B scale: £7,055—,495 p.a., according to qualificaons and experience.

Further particulars may be tained from the Senior Assistt Registrar (Establishments), he University, 6 Kensington errace, Newcastle upon Tyne, EL 7RU, with whom applicaons (3 copies), together with the mes of 3 referees, should be dged not later than 20th March 87.

(2215)E

UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL FELLOWSHIPS IN CANCER MOLECULAR/CELL BIOLOGY

Two posts available to study mechanisms by which oncogenes of src family disrupt microfilamentous actin (Exp Cell Res 165 (1986) 216). Projects (1) analysis of structure/function relationships in cytoskeletal proteins vinculin/α-actinin/talin via cDNA cloning; J Biol Chem in press (2) genomic organization and role of RNA splicing in generating isoforms (3) regulation of expression of the genes.

Applicants should have a PhD in Biochem/Genetics. 1 post cloning experience desirable; 1 post protein biochemist/cell biologist. Available April 1st 1987 for 3 yrs. Salary £8020–10865. CV's with names of 2 referees to Dr Critchley, Dept Biochem, University of Leicester, LE1 7RH.

(2226)E

UNIVERSITY OF BIRMINGHAM

FACULTY OF MEDICINE AND DENTISTRY

DEPARTMENT OF ENDOCRINOLOGY

POSTDOCTORAL FELLOWSHIP

available for research on the structure and regulation of the human thyroxine-binding globulin gene in subjects with congenital deficiency or excess of circulating TBG. Preference will be given to candidates with a background in molecular biology.

The post is available for 3 years and is financed by the Wellcome Trust. Salary on scale £8,020—£12,780, with superannuation; maximum starting £9,495.

Informal enquiries to Dr D.B. Ramsden, 021-472-1311 ext 3802. Applications (3 copies) with names and addresses of two referees to: Assistant Registrar, The Medical School, Birmingham B15 2TJ, from whom further particulars are available by 20 March 1987. Quote reference RF/End/DBR. An Equal Opportunities Employer.

(2166)E

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF CHEMISTRY

Applications from physical chemists or physicists are invited for a Research Fellowship to be held in the research group of Professor A. Carrington, F.R.S. The Fellow will take part in the organisation and operation of the laboratory. Present research is concerned with the spectroscopy of molecular ion beams and some experience with spectroscopy, electronics, lasers, computing or ion beam techniques would be valuable. The post is available for an initial period of 3 years, with the possibility of further renewal. The post is available from 1 August, 1987.

Salary on scale £8,020—£12,780 per annum (award pending).

Applications, (in duplicate) giving date of birth, details of qualifications and experience, together with the names and addresses of two referees, should be sent to: Mr H.F. **Watson, Staffing Department, The University, Southampton SO9 5NH** no later than 31 March 1987. Please quote ref. no. 100/DASC/NA.

(2182)E

Bionetics Research, Inc.–Basic Research Program
NCI–Frederick Cancer Research Facility

THIRD ANNUAL MEETING ON ONCOGENES

July 7–11, 1987

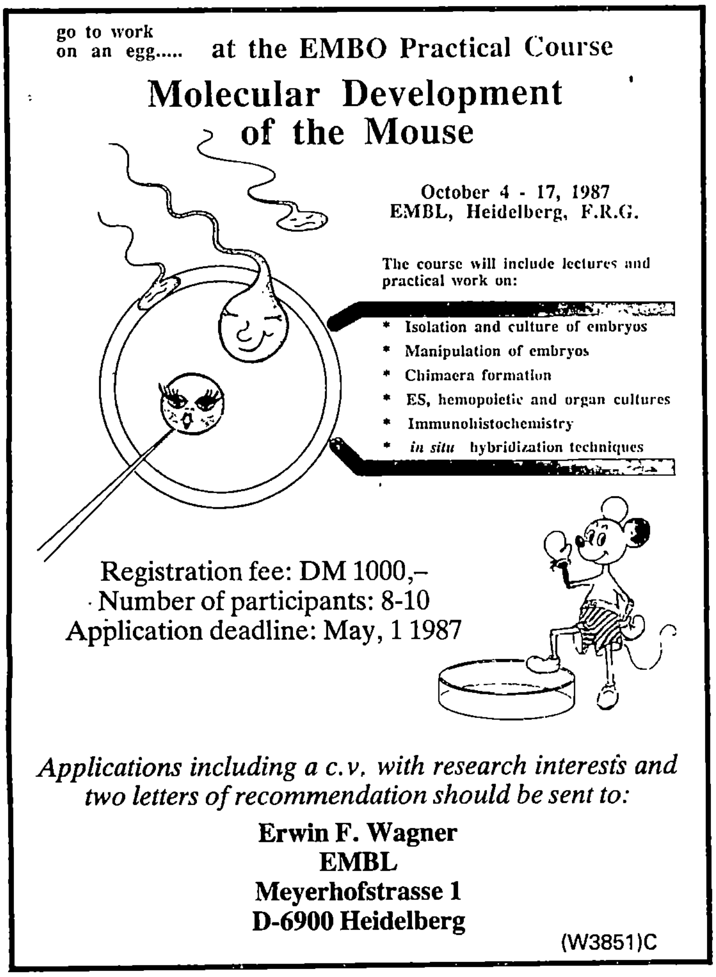
Hood College
Frederick, Maryland

Meeting organizers:

Dr. Stephen Hughes & Dr. Hidesaburo Hanafusa

For information contact:

Ms. Margaret Fanning
Conference Coordinator, PRI
NCI–Frederick Cancer Research Facility
Frederick, Maryland 21701-1013 (NW561)C



go to work on an egg..... at the EMBO Practical Course

Molecular Development of the Mouse

October 4 - 17, 1987
EMBL, Heidelberg, F.R.G.

The course will include lectures and practical work on:

- Isolation and culture of embryos
- Manipulation of embryos
- Chimaera formation
- ES, hemopoietic and organ cultures
- Immunohistochemistry
- *in situ* hybridization techniques

Registration fee: DM 1000,–
Number of participants: 8-10
Application deadline: May, 1 1987

Applications including a c.v. with research interests and two letters of recommendation should be sent to:

Erwin F. Wagner
EMBL
Meyerhofstrasse 1
D-6900 Heidelberg

(W3851)C

KING'S COLLEGE LONDON (KQC) UNIVERSITY OF LONDON

MSc DEGREE IN GENERAL BIOCHEMISTRY

Both part-time and full-time courses will commence in October 1987 and will be held at the Campden Hill Site, Kensington. The part-time course extends over two academic years and involves attendance for one afternoon and one evening per week. The full-time course extends over one academic year and involves a research project. Scholarships may be available for applicants (full-time only) from developing Commonwealth countries who show evidence of high academic calibre. Some bursaries may also be available for part-time applicants.

Qualifications: Candidates should normally hold a first or second class honours degree (or an approved equivalent qualification) in a science subject. Graduates in Medicine, Dentistry or Veterinary Medicine are also admitted. The course is inappropriate to recent graduates from honours Biochemistry courses.

For further information and application forms write, in the first instance, to the Secretary, Biochemistry Department, King's College London (KQC), Atkins Building, Campden Hill, London W8 7AH. (2200)C

UNIVERSITY OF ABERDEEN

MSc/DIPLOMA IN MARINE AND FISHERIES SCIENCE

Applications are invited for a full-time postgraduate course leading to the degree of MSc (12 months or the award of a Diploma (months) in Marine and Fisheries Science.

This interdepartmental cours offered by the University in co junction with the DAFS Marine Laboratory, Aberdeen, and wit participation of the Torry Research Station. The course teache a scientific approach to the exploitation and management o the marine environment and its living resources.

Two terms of formal instruction are provided, on a modular basis, followed by an extended period of research training and practical experience with an active research team (MSc). The range of topics offered and facilities available for the research training element is particularly wide and this aspec the course can be tailored to s the needs of those who wish acquire first hand experience particular techniques.

For further details and app tion write to Head of Departr Department of Zoology, U sity of Aberdeen, Tilly Avenue, Aberdeen AB9 2T (222

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ZOOLOGY

Applications are invited from those who have or who expect soon to obtain a good honours degree and who wish to undertake research training leading to a PhD degree in the following areas:

Title of Project: Cephalopod neurobiology (Supervisor) Dr P.R. Boyle.

Parasite chemotherapy and pathophysiology (Dr L.H. Chappell).

Amine pathways to arthropod inter-neurones (Dr P.J. Fraser).

Carnivore and rodent physiological ecology (Dr M.L. Gorman).

Protein metabolism and energetics (Dr D.F. Houlihan).

Clonal analysis of amphibian development (Dr G.J. MacMillan).

Insect-Plant secondary pound interactions (Dr A. due).

Insect neurobiology (F Mordue).

Foraging theory and po dynamics (Dr J.G. Ollaso

Environmental physiology of fis (Dr I.G. Priede).

Interleukin production b lymphocytes (Mr G. Pugh Humphreys).

Energetics of reproduction i insectivores (Prof. P.A. Racey).

Geographic variation and ec logical adaptation in lizards (D R.S. Thorpe).

Please write in the first instanc to The Head of Department o Zoology, University of Aberdeen Tillydrone Avenue, Aberdee AB9 2TN, enclosing a full c.v. an specifying research area c interest. (2224)

SPECIAL ANNOUNCEMENT

"The Global Impact of AIDS"

International conference to be held at the Barbican Centre, London, England on March 8-10 1988. Organised on behalf of the London School of Hygiene and Tropical Medicine in association with the World Health Organisation. (2203)C

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nature

— the professionals' choice

EUROPEAN Molecular Biology and Biotechnology Summer Workshop

WHEN:
June 29-July 11, 1987

WHERE:
University of Paris VII
Paris, France

FACULTY:

Dr. Steven A. Williams
New England Biolabs, Beverly, Massachusetts; Dept. of Biological Sciences, Smith College, Northampton, Massachusetts; and Program in Molecular and Cellular Biology, University of Massachusetts

Dr. Molly Fitzgerald-Hayes
Dept. of Biochemistry and Program in Molecular and Cellular Biology, University of Massachusetts

Dr. Barbara Osborne
Dept. of Veterinary and Animal Sciences and Program in Molecular and Cellular Biology, University of Massachusetts

TO APPLY
for this workshop please submit a recent curriculum vitae and a brief statement of motivation to:

Dr. Steven A. Williams
c/o Ozyme
31 Rue des Fougeres
Maurepas
78310 France

PRESENTED BY: New England Biolabs Foundation
University of Paris VII
Institut Jacques Monod

SPONSORS: New England Biolabs, Beverly, MA
Ozyme, Maurepas, France

We are pleased to announce the first annual European Molecular Biology and Biotechnology Summer Workshop to be held at the University of Paris VII, Paris, France, from June 29-July 11, 1987.

INTENSIVE BENCH EXPERIENCE. This intensive, two week course emphasizes hands-on molecular biology laboratory work. About six hours each day will be spent working at the bench.

EXPERIMENTS WILL INCLUDE: purification of DNA, restriction enzyme digestion, gel electrophoresis, construction of recombinant DNA molecules, cloning in plasmid and phage vectors, cloning strategies, bacterial transformation and transfection, Southern and Northern transfer and hybridization, radioactive labeling of DNA, and DNA sequencing. Lectures, seminars and discussion sessions (at least two hours each day) will deal with all of the above topics and how these methods are applied in molecular biology research. NOTE: all instructions will be in English.

INTENDED FOR BEGINNERS IN MOLECULAR BIOLOGY. No previous experience in molecular biology is required or expected. Thirty participants will be selected. All instruction will be in English.

FEE: 10000 F per participant includes lab manual and use of all equipment and supplies. Special rates for academic applicants according to availability. A variety of relatively inexpensive hotels is available in the University of Paris VII area.

(NW548)C

APPLICATIONS MUST BE RECEIVED BY April 25th, 1987. Notification of acceptance will be mailed by May 16th, 1987. Payment in full will be due by June 1st, 1987. For additional information, please contact Martin Lauriot-Prevost, Ozyme, (1)30.64.72.34

New England Biolabs Foundation
P.O. BOX 413, WENHAM, MASSACHUSETTS 01984 (617) 468-1833

THE ROYAL VETERINARY COLLEGE
University of London

MSc IN LABORATORY ANIMAL SCIENCE

The College offers a full-time course of study over one calendar year leading to an examination for the degree of MSc in Laboratory Animal Science. In addition, there is provision in the regulations to allow individuals in full-time employment to acquire the degree by part-time attendance and the accumulation of credits over the period of 2, 3 or 4 years. The next course begins in October 1987 and is based at the College premises in London NW1.

The course is intended primarily for veterinary or medical graduates seeking to specialize in all aspects of care, maintenance and use of laboratory animal colonies for biological, biomedical and allied studies. Graduates in biological sciences may also be admitted if they have had practical, full time experience of aspects of laboratory animal science since graduation.

In some cases financial assistance can be provided to meet tuition fees and living expenses.

The course has been specifically designed to fulfil the requirements laid down by the Animals (Scientific Procedures) Act 1986 which has recently come into force. Veterinary practitioners wishing to attend certain aspects of the course without enrolling for the degree are also invited to apply. Details of the syllabus are available on request and a daily tuition fee will be charged.

Further information and application form obtainable from the Registrar, The Royal Veterinary College, Royal College Street, London NW1 0TU. (2192)C

KING'S COLLEGE LONDON

(KQC)

UNIVERSITY OF LONDON

STUDY HISTORY AND PHILOSOPHY OF SCIENCE AT KING'S

One year MSc courses begin October 1987. Facilities for MPhil and PhD by research. Applicants must possess a first degree in one of the natural sciences or mathematics. All courses approved for British Academy grant awards. Early application is advised since the closing date for applying for a British Academy award is 1 May.

Details from: Dr M. Machover, Department of History and Philosophy of Science, King's College London (KQC), Chelsea Campus, Manresa Road, London SW3 6LX. Tel: 01-351 2488, ext 2369. (2201)C

WORKSHOP

THE MIND BROAD ADVENTURE CON

with

THE BIOTECHNOL DIVISION OF FISH SCIENTIFIC

sponsoring

April 14, 1987:

"A View of Biotechnology the Side Lines: Restr enzymes, gene juggling, virus and related topics" t held at the Hyatt Cambrid Boston, Massachusetts,

and

April 21, 1987:

"Molecular Self-Defense: A at contemporary immunolo research and medicine" tc held at the Pooks Hill Marrio Bethesda, Maryland. For fur details write to: S. Da Makover, Ph.D., Fisher Scient 711 Forbes Avenue, Pittsb PA15219 or call (412) 562 170

(NW530)

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A comprehensive series of lectures presenting an integrated intensive course on the near space radiation environment and its effects on man in space. Topics include: radiobiology, dosimetry and magnetospheric physics. Limited number of contributed papers will be considered for presentation. Partial support for travel and living expenses will be available.

Further information contact:

Dr P. McCormack, MD, PhD.
Division of Life Sciences (CODE EB)
NASA, HQ
Washington, D.C. 20546 USA
Telephone 202 453–1543

DEADLINE April 15, 1987 (NW552)C

UNIVERSITY OF EDINBURGH

MSc IN NEUROSCIENCE

The Department of Veterinary Preclinical Sciences at Edinburgh University offers a 1 year MSc course in Neuroscience. The programme is practically based and includes instruction on; single unit recording using intracellular and extracellular recording; voltage-clamp and patch-clamp; intracellular dye injection; brain slice preparations; iontophoresis and immunocytochemistry.

Applications or requests for further information please write to: Dr R J Martin, Department of Preclinical Sciences, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh EH9 1QH. (2209)C

Fourth Annual Nabrit Symposium in the Biomedical Sciences:

Gene Structure, Replication, and Expression

Sponsored by Atlanta University and the National Institutes of Health. 9–11 April 1987, Atlanta, Georgia. Addresses, papers, posters concerned with gene structure, replication, expression, and analyses of gene products.

For information: **Prof. W. Dashek, Nabrit, Symposium Organizing Committee, Atlanta University, 223 Brawley Drive, SW, Atlanta, GA 30314; (404) 681–0251.**

(NW553)M

SEMINARS & SYMPOSIA

CALL FOR POSTERS

The Center for Alternatives to Animal Testing at the John Hopkins School of Public Health is sponsoring its fifth annu international symposium:

PROGRESS IN *IN VITRO* TOXICOLOGY

This symposium will be held in Baltimore on November 4- 1987. We encourage the submission of abstracts for post presentations. Posters of academic endeavours and orga izations offering products of importance to *in vitro* studies encouraged.

If you wish to receive instructions for poster presentatio would like to be placed on the CAAT mailing list, p contact: **Mrs Sheila Kuhn, The Johns Hopkins Cent Alternatives to Animal Testing, 615 North Wolfe St Baltimore, MD 21205, Tel. 301-955-3343.**

Deadline for submission of poster abstracts — October 1987. (NW535)M

THIRD INTERNATIONAL SYMPOSIUM OF THE NORTHERN EYE INSTITUTE

SEEING CONTOUR AND COLOUR

9 — 13th AUGUST 1987

MANCHESTER, ENGLAND

The symposium will adopt a multi-disciplinary approach to question concerning the physiological and psychological basis of visual pe ception. Sessions will cover post-receptoral processing mechanisms; psychophysics of normal and abnormal visual performance; electro- physiological correlates of human visual performance; theoretica models and their application. The proceedings will be published.

The meeting is a satellite symposium of the Second World Congress of Neuroscience 1987 in Budapest.

Invited contributors will include: G B Arden, J Bullier, W Burke, A Damasio, K K DeValois, B Dreher, D H Foster, P Gouras, P Hammond, D H Hubel, A Jeffreys, J H Kaas, H Kennedy, P E King-Smith, D J Laming, B Lee, M Livingstone, C R Michael, W H Merrigan, K T Mullen, D Perrett, E Pöppel, D Regan, M Rizzo, E Rolls, K Ruddock, A Sillito, Spekreijse, H G Sperling, S Thorpe, D J Tolhurst, T R Vidyasaga Zeki.

Full details from: Dr C M Dickinson (NEI87), Department of Op mic Optics, UMIST, PO Box 88, Manchester, M60 1QD, UK. Te 236 3311 ext 2550 Telex: 666094. (2242)

1st International Symposium
on
POST-TRANSLATIONAL MODIFICATIONS OF PROTEINS and *AGEING*

Lacco Ameno d'Ischia
Bay of Naples, Naples (Italy)
May 11 - 15, 1987

Under the Auspices
of
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The Italian Society of Biochemistry

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Topics

1. *Transglutaminase-Mediated Reactions:*

Cellular and Extracellular Transglutaminases; Enzyme Expression; Factor XIII and Blood Plasma Proteins; Keratinocyte Differentiation; Protein Cross-linking and Cell Ageing: the Erythrocyte and the Lens Models; Senile Cataract Pathogenesis; Modulation of Cellular Antigenicity; Suppression of Sperm Immunogenicity.

2. *Protein Methylation:*

A. *Enzymatic Methyl Esterification: Methylation of Proteins and Ageing; Substrate Specificity. Effect of Purine Analogs; Substrates in Alzheimer Tangles and Plaques.*
B. *N-Methylation of Proteins: Cytochrome C as a Substrate of Protein Methylase III: Methylation of Arginine Residues in Myelin Basic Protein; Structural Modifications of Calmodulin; Histone Methylation and Modulation of Gene Expression.*

3. *Protein Phosphorylation:*

Protein Kinase C; Tyrosine Protein Kinases Associated with Retroviruses; Tyrosine Protein Kinases Associated with Hormone Receptors; Regulation of Adenylate Cyclase; Phosphoprotein Phosphatases.

4. *Other Post-Translational Modifications:*

Histone Acetylation; ADP-Ribosylation of Proteins; Protein Glycosylation; Limited Proteolysis.

Invited Speakers

D.W. Aswad *(Irvine, USA)*; F. Auricchio *(Naples, Italy)*; J.L. Benovic *(Durham, USA)*; P.J. Birckbichler *(Ardmore, USA)*; G. Carpenter *(Nashville, USA)*; A. Cerami *(New York, USA)*; S.I. Chung *(Bethesda, USA)*; S. Clarke *(Los Angeles, USA)*; P. Cohen *(Dundee, UK)*; P.K.A. Davies *(Houston, USA)*; W.W. de Jong *(Nijmigen, The Netherlands)*; J.A. Duerre *(Grand Forks, USA)*; L. Fesus *(Debrecen, Hungary)*; C. Gagnon *(Montreal, Canada)*; P. Galletti *(Naples, Italy)*; S. Kim *(Philadelphia, USA)*; E.G. Krebs *(Seattle, USA)*; L. Lorand *(Evanston, USA)*; G. Man *(Miami, USA)*; J. Mc Donagh *(Boston, USA)*; E. Melloni *(Genoa, Italy)*; D. Mosher *(Madison, USA)*; A. Mukherjee *(Bethesda, USA)*; C. O'Connor *(Shrewsbury, USA)*; W.K. Paik *(Philadelphia, USA)*; P.J. Parker *(London, UK)*; L. Pinna *(Padua, Italy)*; S. Pontremoli *(Genoa, Italy)*; R. Porta *(Naples, Italy)*; R. Rice *(Boston, USA)*; J. Roth *(Bethesda, USA)*; S. Shall *(Brighton, UK)*; F.L. Siegel *(Madison, USA)*; J. Stock *(Princeton, USA)*; R.M. Tanguay *(S.te-Foy, Canada)*; G. Thomas *(Basel, Switzerland)*; G. Vidali *(Genoa, Italy)*; H.G. Williams-Ashman *(Chicago, USA)*; V. Zappia *(Naples, Italy)*

Symposium Program: *The Symposium will be organized in sessions of invited lectures devoted to specific topics and poster sessions related to the overall field of post-translational modifications of proteins.*

Proceedings: *The proceedings of the Symposium will be published in a "camera-ready" volume by an International Publishing House. They will include papers upon invitation and a number of selected poster contributions.*

(W3847)M

Cold Spring Harbor Symposium on Quantitative Biology—Volume LII

EVOLUTION OF CATALYTIC FUNCTION

MAY 27 – JUNE 3, 1987

Organized by:
J.D. Watson, Cold Spring Harbor Laboratory

The 52nd Symposium will be devoted to the **Evolution of Catalytic Function** and will focus on the following topics: The Primordial Soup; The Essentials of a Catalytic Molecule; The Anatomy of RNA; RNA as a Catalyst; RNA as a Template; Ribosomes, Protein Synthesis, and the Genetic Code; Proteins: Molecular Dynamics; Proteins: Structure; Proteins: Catalytic Function; Non-Protein Co-factors in Catalysis; Enzymatic and Genetic Components of the Progenote; The Organization of DNA into Exons and Introns; and Evolutionary Trees.

Speakers include:

S. ALTMAN
R. BALDWIN
P. BARTLETT
M. BELFORT
S. BENKOVIC
S. BENNER
J. BERG
C. BLAKE
C. BRANDEN
R. BRESLOW
R. CARRELL
T. CECH
C. CHOTHIA
C. CRAIK
T. CREIGHTON
J. DARNELL
W. DAVIES
W. DE GRADO
W.F. DOOLITTLE
R. DOOLITTLE
M. EIGEN
D. EISENBERG
J. FERRIS
A. FERSHT
W. FITCH
P. FREY
S. GERBI
W. GILBERT
M. GO
M. GOODMAN
T. INOUE
W. JENCKS
A. JONES
G. JOYCE
T. JUKES
E. KAISER
M. KARPLUS
A. KLUG
J. KNOWLES
D. KOLAKOFSKY
D. KOSHLAND
J. KRAUT
R. KRETSINGER
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T. MIYATA
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D. MORAS
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L. ORGEL
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H. OZEKI
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A WEINER
J. WELLS
G. WERTZ
F. WESTHEIMER
A. WILSON
H. WITTMANN
A. YONATH

Registration forms may be obtained from:
Meetings Coordinator
P.O. Box 100, Cold Spring Harbor, NY 11724
(516) 367-8346

(NW549)M

NATO/EMBO/FEBS

International Summer School on Molecular and Cell Biology

The Spetsai Summer School, 1987, sponsored by NATO, EMBO and FEBS will be held in Spetsai, Greece, from August 30th to September 12th, 1987. The subject will be "Molecular Biology of Development". Lectures, discussions, and tutorials in English will cover the following topic areas:

1. **Cell Lineage and Cell Fate**
2. **Developmental Genes**
3. **Somatic Differentiation**
4. **Tissue-specific Gene Regulation**
5. **Plant Development**

The school will be suitable for advanced predoctoral students and for postdoctoral workers in related fields. Lecturers will include:

S. Artavanis-Tsakonas (USA)
D. Baltimore (USA)
T. Caskey (USA)
V. Chapman (USA)
A. Evangelopoulos (Greece)
W. Gehring (CH)
H. Goodman (USA)
P. Gruss (W Germany)
J. Hershey (USA)
R. Jaenisch (USA)
A. McLaren (UK)
G. Morata (Spain)
P. Overbeek (USA)
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R. Phillips (Canada)
J. Sulston (UK)
W. Schaffner (CH)
J. Schell (W Germany)
E. Wagner (W Germany)
D. Wolgemuth (USA)

Applications should be sent to: The Spetsai Summer School Secretary, Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030 USA, no later than April 27, 1987. They should contain a curriculum vitae, including the applicant's scientific background and interests, plus a letter of recommendation. The applicants will be informed by May 25, 1987, whether they have been accepted. A deposit of US $100 will then be due. In general, participants will be required to pay for demi-pension accommodation (an extra US $100) and their travel expenses. A limited number of fellowships are available towards the cost of travel and accommodation in special cases.

(NW531)M

PROPOSALS

CALL FOR RESEARCH PROPOSA

The Center for Alternatives to Animal Testing of the Hopkins School of Public Health is soliciting proposals. research proposals should provide the fundamental knowledge base to develop alternative methods to whole animals for the safety evaluation of commercial products.

The Center encourages the development of methods using human cells/cell lines in addition to other *in vitro* approaches to toxicity evaluation. Funds are available for studies of skin and eye irritation, inflammation, acute toxicity and other organ specific toxicity. At present, funds are unavailable for mutagenicity and carcinogenicity.

Guidelines for preparation of proposals can be obtained from **Mrs Joan Poling, The Johns Hopkins Center for Alternatives to Animal Testing, 615 North Wolfe Street, Baltimore, MD 21205. Tel. 301-955-3343.** Deadline for submission of research proposals — May 29, 1987.

(NW536)X

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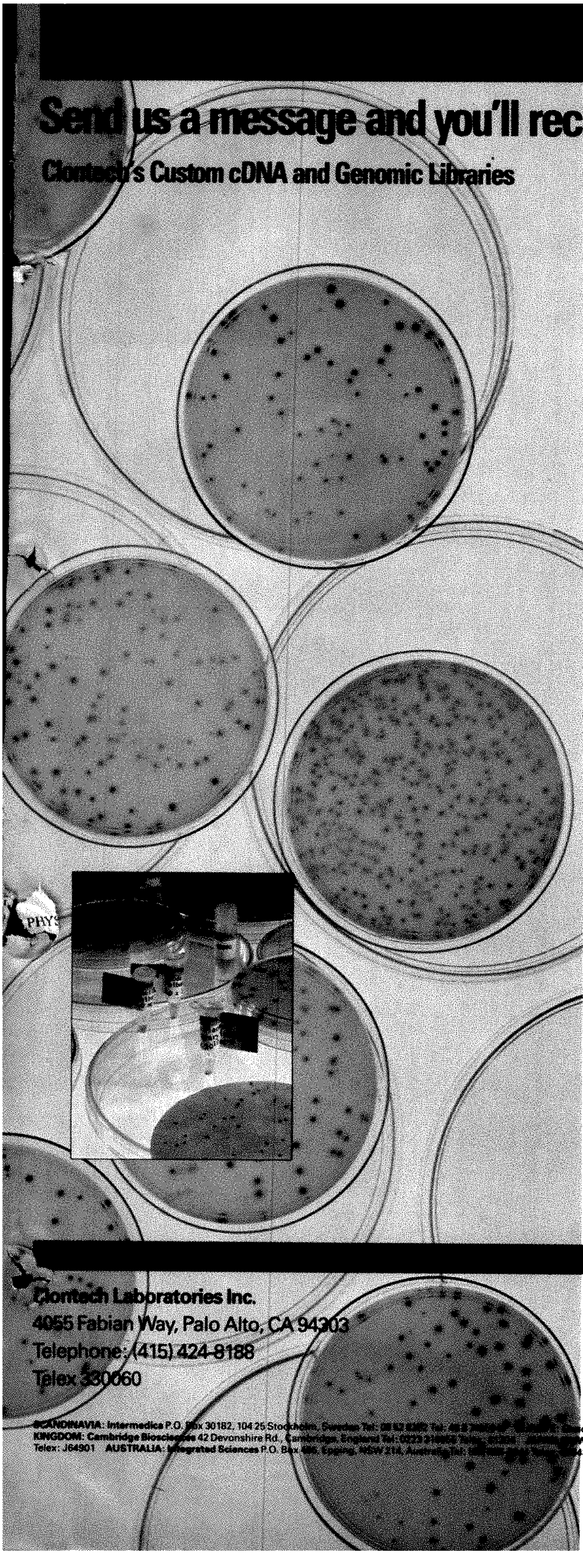

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